REPORT

of

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., Inc.

1986

Organization and Policy

The Council for Tobacco Research — U.S.A., Inc. is the sponsoring agency of a program of research into questions of tobacco use and health. It is the outgrowth of an organization formed early in 1954 by tobacco manufacturers, growers and warehousemen. Research support has been mainly through a program of grants-in-aid supplemented by contracts for research with institutions and laboratories. The Council does not operate any research facility.

The Scientific Advisory Board to The Council meets regularly to evaluate applications for research support, judging them solely on the basis of scientific merit and relevance.

The Council awards research grants to independent scientists who are assured complete scientific freedom in conducting their studies. Grantees alone are responsible for reporting or publishing their findings in the accepted scientific manner — through medical and scientific journals and societies.

WILLIAM D. HOBBS
Chairman

1986 REPORT

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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
900 Third Avenue, New York, N.Y. 10022

SCIENTIFIC ADVISORY BOARD

The Council for Tobacco Research - U.S.A., Inc.
as of December 31, 1986

CONTENTS

Introduction

The abstracts in this annual report bring to at least 3,098 the number of published scientific documents acknowledging Council support. The Council has been funding studies in smoking and health by independent researchers for 32 years.

Two distinguished scientists joined the Scientific Advisory Board to The Council during 1987. They were Jeffrey K. Aronson, Ph.D., Reader in Pharmacogenetics and Wellcome Trust Senior Lecturer in the Department of Pharmacology at St. Mary's Hospital Medical School, London, England, and Alfred J. Knudson, Jr., M.D., Ph.D., who recently retired as Director of the Institute for Cancer Research in Philadelphia and is now affiliated with its Fox Chase Cancer Center.

Also during the year, Roswell K. Boutwell, Ph.D., rejoined the Board after spending two years in Hiroshima as Chief of Research of the Radiation Effects Research Foundation. He has returned to his former institution, the McArdle Laboratory of Cancer Research at the University of Wisconsin in Madison.

Peter M. Howley, M.D., who had been a Board member since 1982, resigned during the year.

Blanton C. McAllister, Jr., Ph.D., who joined the Council's scientific staff in 1984 as an Associate Research Director, was promoted to Research Director.

More than $110,000,000 has been made available by The Council since 1954; this year it was established, for research by 542 scientists for 986 original projects in 296 medical schools, hospitals and research institutions.

Abstracts of Reports

[illegible] abstracts approved by the authors of reports on new research and [illegible] from the Council that have appeared in scientific journals [illegible] Annual Report. The name of the grant recipient is in italics.

The abstracts are grouped under these headings: I. Cancer-Related Studies; II. Biochemistry; III. Heart and Circulation; IV. Neuropharmacology and Psychiatry; V. Physiology and Biochemistry; VI. Immunology and Adaptive Mechanisms; VII. Molecular Studies; VIII. Epidemiology.

I. *Cancer-Related Studies*

IDENTIFICATION OF PROCESSING EVENTS IN THE SYNTHESIS OF PLATELET-DERIVED GROWTH FACTOR-LIKE PROTEINS BY HUMAN OSTEOSARCOMA CELLS

The human osteosarcoma-derived cell line U-2 OS expresses c-*sis* mRNA and synthesizes platelet-derived growth factor (PDGF)-like proteins. Pulse–chase experiments [illegible] the proteins of 23 kDa and 18 kDa are synthesized first. The 23-kDa protein undergoes dimerization and proteolysis, giving rise to the 30-kDa dimeric protein secreted by the cells. The 18-kDa protein is proteolytically cleaved in a complex series of steps that give rise to several intracellular species. It is also the likely precursor of high molecular mass PDGF-like or PDGF-associated proteins secreted by these cells. The processing and secretion of the 18-kDa protein is slower than that of the 23-kDa protein. Subcellular fractionation and studies with the antibiotic monensin indicate that these processing events occur in the Golgi and endoplasmic reticulum compartments of U-2 OS cells.

G[illegible] E. A. Owen, A. F. Williams, S. R. [illegible], and *Avrum Ziff*, M.D.

[illegible] of the National Academy of Sciences—USA 83:4636–4640, July 1986

Other support: National Institutes of Health and the American Cancer Society.

Ludwig Institute for Cancer Research and Department of Nutrition, Harvard School of Public Health, Boston.

HUMAN T-LYMPHOCYTES SYNTHESIZE AND SECRETE PROTEINS RELATED TO PLATELET-DERIVED GROWTH FACTOR

Human B-lymphocytes in culture (HL-60) synthesize and secrete proteins that are recognized by antiserum to human platelet-derived growth factor (PDGF). The molec-

ular mass of the intracellular proteins immunoprecipitated by PDGF-related proteins were also identified in the conditioned medium of the cells. Several of these immunoprecipitated proteins were glycosylated. A single protein of 46 kDa was immunoprecipitated from the cell-free translation products of mRNA obtained from the leukemia cells. Antiserum to the C but not to the N terminus of the predicted amino acid sequence of the transforming protein p28^{sis} PDGF-2 also immunoprecipitated proteins secreted by the HL-60 cells. These findings provide a direct demonstration for the synthesis and secretion of PDGF-like proteins by leukemia cells in culture. These proteins do not appear to be coded by the known c-*sis*/PDGF-2 locus since no *sis* mRNA was detectable in the HL-60 cells.

Pantazis, P., Lanthanoff, L., Pelicci, P. G., and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences, USA **83**, 5526–5530, August 1986

Other support: National Institutes of Health

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston.

INDUCTION OF c-*sis* GENE EXPRESSION AND SYNTHESIS OF PLATELET-DERIVED GROWTH FACTOR IN HUMAN MYELOID LEUKEMIA CELLS DURING MONOCYTIC DIFFERENTIATION

Phorbol esters induce the differentiation of human myeloid leukemia cells HL-60 and U-937 along the monocyte-macrophage lineage. This process has been associated with the induction of several cellular protooncogenes, including the c-*fos* and c-*fms* genes. We now report that phorbol ester-induced differentiation of the HL-60 and U-937 cells results in the induction of the expression of the c-*sis* platelet-derived growth factor 2 (PDGF-2) protooncogene. *sis* mRNA transcripts were not detectable in the uninduced cells but were detectable within 12 hr of phorbol ester induction. Concomitantly, the induced cells were shown to synthesize and secrete biologically active PDGF-like proteins, identified in the conditioned medium of the phorbol ester-treated cells by direct immunoprecipitation with PDGF antiserum. Addition of cycloheximide to phorbol ester-treated HL-60 cells superinduced *sis* mRNA transcripts. c-*sis* gene transcripts were also detected in freshly isolated human monocytes but not in human granulocytes or in HL-60 cells induced to differentiate along the granulocytic lineage. Activation of the c-*sis*/PDGF-2 gene in human hematopoietic cells during monocytic differentiation may serve in the mediation of physiologic functions of the differentiated cells by means of the secretion of potent PDGF-like mitogens.

Pantazis, P., Sutherland, E., Kufe, D., and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences, USA **83**, 6455–6459, September 1986

Other support: National Institutes of Health

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston.

HIGH DENSITY LIPOPROTEINS DECREASE BENZO[A]PYRENE DIOL EPOXIDE AND METABOLITE KEY OF (+)-7,8-DIHYDROXY-9,10-EPOXY-7,8,9,10-TETRAHYDROBENZO[A]PYRENE IN V79 CHINESE HAMSTER CELLS

The effects of separate lipoproteins or of serum with high or low lipoprotein concentration on formation of lipophilic carcinogen adducts with DNA and on mutagenicity of the carcinogen was investigated using V79 Chinese hamster lung cells. Binding of (+)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) to DNA and BPDE induction of 6-thioguanine resistant mutants in V79 cells was significantly lower after 0 or 4 h when the medium was supplemented with purified BPDE and was lower after 1 h but not 4 h when the medium was supplemented with serum containing a high concentration of high density lipoprotein (HDL). Cells grown in medium with no serum or HDL supplementation exhibited the highest levels of both BPDE-DNA adduct formation and mutagenesis after 0 or 4 h. Cells exposed to BPDE in HDL-supplemented medium showed decreased adduct formation and mutagenesis when compared to cells treated with BPDE in PBS-supplemented medium. After 4 h, cells treated with BPDE in HDL-supplemented medium gave the highest levels of adduct formation and the highest mutation frequencies. These results suggest that both HDL and HDL effectively decrease the concentration of BPDE available to V79 cells exposed to this mutagen for short periods of time, resulting in decreased interaction of BPDE with DNA and decreased BPDE-associated mutagenesis. Not only was BPDE-DNA adduct formation and mutagenesis increased as a function of increased exposure time in the presence of HDL. The results suggest that HDL or some HDL apoprotein(s) may sequester and/or bind BPDE, thereby protecting cells and DNA from BPDE damage. V79 cells also function at less

Yoon, J. S., Ho, N. W. Y., Ji, G. D., Lee, C. O., and Evans, F. E.

Mutat. Res., vol. **159**, no. 5, pp. 205–211, 1986.

Other support: National Institutes of Health, the Texas Agricultural Experiment Station, Texas A and M University, and the U. S. Department of Agriculture.

From the Departments of Anatomy and Biochemistry and Biophysics, College of Veterinary Medicine, Texas A and M University, College Station, and Veterinary Toxicology and Entomology Research Laboratory, U. S. Department of Agriculture, College Station, TX.

PHOSPHATIDYLINOSITOL-DEPENDENT ACTIVATION OF DNA POLYMERASE ALPHA

DNA polymerase alpha was activated in vitro by a 55,000 dalton phosphatidylinositol-dependent protein kinase (PIK) from rat liver. PIK phosphorylated phosphatidylinositol (PI) and the greatest percentage of incorporation was with PI. PIK kinase and ATP-activated DNA polymerase alpha were separated by DEAE-cellulose chromatography. DNA polymerase alpha was directly activated by phosphatidylinositol 4-phosphate (PIP) and by the addition of PIP kinase and ATP. Activation of DNA polymerase alpha by PIP kinase and PIP was

demonstrated using [illegible] as the phosphate donor. [illegible] with phosphatidylinositol produced [illegible] kinetics of enzyme activation suggesting that activation occurs [illegible] enzyme to DNA template primer. These data indicate that DNA polymerase alpha may be activated directly in the presence of protein kinase, ATP, and phosphatidylinositol and suggest that phosphorylation of the enzyme may constitute a [illegible] mechanism of enzyme activation.

Sylvia, V. L., Jr., C. O., Norman, J. O., Curtin, G. M., and Boyle, D. J.

Biochemical and Biophysical Research Communications **135**: [illegible]

Other support: National Institutes of Health, U. S. Department of Agriculture, and the Texas Agriculture Experiment Station

From the Department of Anatomy, Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station; Department of Biology, Korea Institute of Technology, Republic of Korea; and Veterinary Toxicology and Entomology Research Laboratory, U. S. Department of Agriculture, College Station.

INHIBITION OF DNA POLYMERASE ACTIVITY BY METHYL METHANESULFONATE

Methyl methanesulfonate (MMS) inhibits both thymidine incorporation into DNA in mitogen-activated human lymphocytes and deoxythymidine triphosphate incorporation into template DNA by DNA polymerases in a cell-free system. When MMS-modified DNA was used as the template for DNA synthesis utilizing [illegible] DNA polymerase α, nucleotide incorporation into template DNA was [illegible]. When unmodified DNA was used as the template for DNA synthesis utilizing MMS-modified DNA polymerase α, nucleotide incorporation was differentially [illegible] depending on the MMS concentration. An analysis of the kinetics of DNA polymerase α inhibition showed that incorporation of [illegible] deoxynucleoside triphosphates into DNA [illegible] was non-competitively inhibited by MMS, which is consistent with [illegible] of MMS modification of the enzyme. These data indicate that MMS modification of DNA [illegible] DNA polymerase α [illegible] incorporation of deoxynucleoside triphosphates into template DNA *in vitro*. The data further indicate that [illegible] both DNA polymerase α and DNA template synergistically decreases inhibition of DNA synthesis.

Norman, J. O., Joe, C. O., and Boyle, D. J.

Mutation Research **165**: 79, 1986.

Other support: National Institutes of Health, Texas Agricultural Experiment Station, and the U. S. Department of Agriculture.

From the Veterinary Toxicology and Entomology Research Laboratory, U. S. Department of Agriculture, College Station, TX, and Departments of Anatomy, Physiology, College of Veterinary Medicine, Texas A and M University, College Station.

EXPRESSION AND CHROMOSOMAL LOCALIZATION OF THE CYTOCHROME P-450 GENE IN HUMAN MITOGEN-STIMULATED LYMPHOCYTES

Genetic differences in aryl hydrocarbon hydroxylase (AHH) (flavoprotein linked monooxygenase, EC 1.14.14.1) activity in cultured lymphocytes have been linked with individual risk for certain environmentally caused cancers. Cytochrome P-450 is the form of cytochrome P-450 most closely associated with AHH activity. In this study the chromosomal localization and the expression of human cytochrome P-450 gene were determined in phytohemagglutinin-stimulated lymphocytes. *In situ* hybridization analysis provided assignment of the structural gene for human cytochrome P-450 to chromosome 15 (q22-q24). Treatment of lymphocytes with benzanthracene increased the amount of mRNA hybridized to the cloned cytochrome P-450 gene. The level of cytochrome P-450 mRNA in these lymphocytes correlates well with the induced AHH activity, indicating that not cytochrome P-450 enzymes contribute little to the individual differences in the level of AHH activity in the lymphocytes. Southern analyses of genomic DNA from individuals with high and low induced AHH activity demonstrated no detectable differences in the pattern or intensity of restriction fragments after treatment with benzanthracene from either individual. This finding, together with the excellent correlation between the induced cytochrome P-450 and AHH activity, suggests that transcriptional control rather than gene amplification or gross forms of gene rearrangement are responsible for cytochrome P-450 induction in man. Measurements of cytochrome P-450 mRNA content in cultured lymphocytes provide an alternative approach to the assay of AHH activity in assessing AHH phenotype and predicting differential susceptibilities to deleterious environmental agents.

Aoyama, T., Nebert, D. W., Swanson, D. C., and Gonzalez, F. J.

Cancer Research **46**: 2432–2437, 1986.

Other support: North Carolina United Way.

From the Department of Pediatrics, Duke University Medical Center, Durham, NC.

GLYCOLIPID ANTIGEN EXPRESSION IN HUMAN LUNG CANCER

Several monoclonal antibodies which recognize carbohydrate sequences distinguish between different types of human lung cancer immunologically. These antibodies probably recognize glycolipid antigens produced by the cancer cells. When glycolipids are separated by thin-layer chromatography, immunostaining of the chromatograms yields characteristic patterns of antigen-positive bands. To determine whether glycolipid patterns are useful in the classification of lung cancer, 10 human lung cancer cell lines comprising the major histological types of primary lung cancer were studied. Neutral glycolipids and gangliosides were isolated and separated by thin-layer chromatography. Six anti-carbohydrate antibodies which recognize structurally related antigens were used for immunostaining. Neuraminidase treatment of the chromatograms was used to distinguish sialylated antigens. All the cell lines were unique with regard to the type and relative amount of chromatography pattern of the glycolipid antigens produced. Small cell lung cancer cell lines synthesized the greatest variety of antigens, whereas cell lines with large cell carcinoma synthesized the least. Interestingly, there was an inverse relationship between expression of some glycolipid antigens and DNA

amplification of the c-*myc* oncogene. This suggests that enhanced c-*myc* expression may influence the types of glycolipids expressed at the surface of lung tumor cells.

Spyratos, S. P., Spyratos, P. F., Dubois, C., Malsellin, J., Magnato, J. L., Cattaneo, E., Civon, C. L., Mhuna, J. D., and Ginsburg, V.

Cancer Research **46**:4751-4755, 1986.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; Division of Pediatric Oncology, the Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD; and Department of Medicine, Dartmouth Medical School, Hanover, NH.

LECTIN HISTOCHEMISTRY OF PAPILLARY AND FOLLICULAR CARCINOMA OF THE THYROID GLAND

The lectin-binding properties of human follicular and papillary carcinoma were studied histochemically and compared with lectin binding to normal or goitrous thyroid tissue. Well-differentiated minimally invasive follicular carcinoma showed a lectin-binding pattern essentially identical to those of the normal thyroid gland and benign adenomatous lesions. Overtly invasive follicular carcinoma showed focal reactivity with some lectins that were nonreactive with normal follicular thyroid cells. *Sambucus nigra*, soybean, and *Ulex europaeus* in three of three cases, *Ulex europaeus* in two of three cases, and *Dolichos biflorus*, *Lotus tetragonolobus*, and peanut in one of three cases. In papillary carcinomas, the cells lining the papillary structures reacted focally with some lectins that did not bind to normal thyroid cells (*Sambucus nigra* and *Ulex europaeus* in seven of seven cases, *Helix pomatia*, *Helix aspersa*, and soybean in four of seven cases, and peanut, *Griffonia simplicifolia*, *Dolichos biflorus*, and *Vicia villosa* in one of seven cases). All these lectins, as well as those reacting with normal thyroid cells, reacted more strongly with cells of papillary structures than with those forming solid nests and follicles. Despite these lectin-defined differences in the composition of glycoconjugates of benign and malignant thyroid cells, the inconsistent and focal nature of the changes precludes the use of lectins in diagnostic histopathology.

Submitted for Symposia, Mr. and Date received: [illegible]

Archives of Biochemistry and Biophysics, Vol. [illegible], pp. 720-729, August 1987

From the Department of Pathology, Hahnemann University School of Medicine, Philadelphia

HARVEY *RAS* GENES TRANSFORM WITHOUT MUTANT CODONS, APPARENTLY ACTIVATED BY TRUNCATION OF A 5′ EXON OF EXON II

The hypothesis is tested that the *ras* gene of Harvey sarcoma virus (Ha-SV) and the proto-DNAs from certain tumor cells derive transforming function from specific codons in which they differ from normal proto-*ras* genes. Molecularly cloned Harvey proviral vectors carrying viral *ras*, normal rat proto-*ras*, and recombinant *ras* genes in which the virus-specific *ras* codons 12 and 59 were replaced by proto-*ras* equivalents each transformed mouse 3T3 cells after latent periods that

[illegible] Viruses with or without virus-specific *ras*-coding sequences all transformed [illegible] Studies especially with [illegible] However, in the absence of virus replication, certain cellular *ras* genes were beneficial for transforming function. Deletion of most *ras* regions of Ha-SV did not affect transforming function. We conclude that specific *ras* coding sequences are not necessary for transforming function. Comparisons of the *ras* sequences of Ha-SV, BALB-SV, and Rasheed SV with sequences of proto-*ras* genes from rat and human revealed an upstream proto-*ras* exon, termed exon 1. The 3′ end of this exon is present in all three viruses and in a *ras* pseudogene of the rat. Since *ras* genes without mutations since exon 1 are truncated in viral *ras* genes and all transforming proteins of DNAs of the Harvey and the Kirsten *ras* family, we propose that [illegible] by truncation of exon 1 either via viral transduction or [illegible] to transformation. The proposal implies that truncated proto-*ras* genes [illegible] tumors may not be cellular cancer genes.

Cytogenetic and Cell Genetics, 32: 1-41, 1982

Proceedings of the National Academy of Sciences, USA 83: 2344-2348, 1986

Other support: National Cancer Institute and the Deutsche Forschungsgemeinschaft.

From the Department of Molecular Biology and the Virus Laboratory, University of California, Berkeley.

ARE ACTIVATED PROTO-*ONC* GENES CANCER GENES

Cellular genes which are related to retroviral transforming (*onc*) genes have been termed proto-*onc* genes. As is now widely believed to be potential cancer genes. In some tumors, proto-*onc* genes are mutated or expressed more than in normal cells. Under these conditions proto-*onc* genes are thought to be activated to functional cancer genes. In view of two hypotheses. The one gene-one cancer hypothesis which suggests that one activated proto-*onc* gene, like a viral *onc* gene, is sufficient to cause cancer and the multigene-one cancer hypothesis which speculates that an activated proto-*onc* gene, unlike a viral *onc* gene, is a necessary but not a sufficient cause of cancer. The evidence for these hypotheses is reviewed here using as examples proto-*ras* and proto-*myc*, the cellular prototypes of the *ras* genes of avian sarcoma virus MC29, and Harvey sarcoma virus. Since [illegible] for transforming potentially activated [illegible] consistently associated [illegible] specific tumor and do not [illegible] and since as yet no set of an activated proto-*onc* gene and a [illegible] with transforming [illegible] has been isolated from a [illegible] proto-*onc* genes are sufficient but never necessary [illegible]

Biochimica et Biophysica Acta

In: Cooper, G. and Grossman, A., eds. *CELL TRANSFORMATION*. New York: Raven Press, Cold Spring Harbor Symposia, 1985; pp. 21-63.

Other support: National Cancer Institute.

From the Department of Molecular Biology, University of California, Berkeley, The Salk Institute, San Diego, CA; Laboratory of Molecular Oncology, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD; and Genentech, Inc., South San Francisco, CA.

NECROPSY EVIDENCE OF DETECTION BIAS IN THE DIAGNOSIS OF LUNG CANCER

The correct diagnosis had not been made during life in 26% of 153 patients with lung cancer found in necropsies performed between 1971 and 1982. The likelihood of a correct antemortem diagnosis showed distinctive gradients in relation to the patients' history and amount of cigarette smoking, symptomatic manifestations, and anatomic extensiveness of the cancers. However, cigarette smoking still exerted a diagnostic effect in patients with similar symptoms and similar degrees of anatomic spread. Furthermore, if a lesion was present, chest films were more likely to be radiologically interpreted as cancer in nonsmokers. The results suggest that smokers receive preferential consideration regarding the diagnosis of lung cancer. This detection bias can have adverse scientific consequences in depriving nonsmokers of suitable therapy, inducing false-high estimates of the true magnitude of the smoking-lung-cancer association, and in distracting etiologic attention from other agents that may cause lung cancer.

McFarland, M. L., Beaver, M. R., and Wells, C. K.

Archives of Internal Medicine **146**:1695–1698, September 1986.

Other support: A. W. Mellon Foundation.

From the Department of Medicine and Epidemiology and the Robert Wood Johnson Clinical Scholars Program, Yale University School of Medicine, New Haven, CT.

CLINICAL FEATURES OF LUNG CANCERS DISCOVERED AS A POSTMORTEM "SURPRISE"

Despite improved modern techniques, many patients with primary lung cancer escape detection of their disease during life. In a review of postmortem records at a university hospital, 28% of 153 primary lung cancers found at necropsy had not been diagnosed while the patient was alive. The male:female ratio was 1.3 in this undetected group, compared with 2.3 in the detected group. The main clinical features that seemed to lead to misdiagnosis were: (1) terminal clinical state—patients who were too sick for further diagnostic searches; (2) absence of suggestive primary symptoms—patients who felt well enough to go about showing primary lung cancer; and (3) absence of cigarette smoking. As might be expected, patients with lung cancer at necropsy, the proportion of non-smokers was higher in the previously undiagnosed group than in the group with antemortem diagnoses, even when patients were stratified for primary symptoms. The findings suggest the need for diagnostic alertness to the possibility that curable lung cancer can occur in patients who have a positive chest film lesion but who are non-smokers and who lack typical symptoms.

McFarland, M. L., Beaver, M. R., and Wells, C. K.

JAMA **256**:637–641, 1986.

Other support: A. W. Mellon Foundation.

From the Department of Medicine and Epidemiology and the Robert Wood Johnson Clinical Scholars Program, Yale University School of Medicine, New Haven, CT.

ISOLATION AND PARTIAL CHARACTERIZATION OF VIRUS-TRANSFORMED HUMAN FIBROBLAST CELL LINES REPRESENTING THE A, C AND VARIANT COMPLEMENTATION GROUPS OF XERODERMA PIGMENTOSUM

We have established viral transformed, apparently permanent, immortalized cell lines from skin fibroblasts representative of normal and xeroderma pigmentosum XP-A, C and variant individuals. The XP-C and XP-variant cells represent complementation groups not previously available as permanent lines. All the new permanent cell lines exhibit SV40 T antigen expression. They are all immortalized and have growth characteristics typical of viral transformants. They have retained the phenotypic excision UV sensitivity, reduced repair synthesis and deficient pseudo-repair typical of the XP complementation group they represent. Additional studies, the newly established transformable with the selectable pSV2-gpt-SV40. The XP-C and XP-variant cell lines show enhanced transfection with UV-irradiated plasmid DNA, a phenomenon previously reported for normal human cells and other immortalized cells from the A and D complementation groups of XP.

Barbara De Fabo, Richard A. and Errol C.

The Journal of Molecular and Cellular Biology, 1985 (publication)

Other support: U.S. Department of Energy

From the Department of Pathology, Stanford University School of Medicine, Stanford, CA

NUCLEOTIDE EXCISION REPAIR OF DNA IN EUKARYOTES: COMPARISONS BETWEEN HUMAN CELLS AND YEAST

Little is known about the molecular mechanisms of nucleotide excision repair in eukaryotes. Such repair-deficient cells have been stimulated by the availability of excision repair-deficient cell lines from patients suffering from the autosomal recessive disease, xeroderma pigmentosum. Such studies have contributed appreciably to an understanding of the genetic complexity of excision repair in human cells. However, to date no human excision repair genes or gene products known to directly mediate the repair defect in mammalian cells have been isolated. The yeast *Saccharomyces cerevisiae* has been shown to possess a system for excising pyrimidine dimers from DNA. By exploiting the molecular genetic advantages of this eukaryotic cell, it has been demonstrated that at least nine yeast genes are involved in this pathway and at least five genes are required for the specific incision of DNA containing pyrimidine dimers. These five genes have been cloned by complementation of the corresponding mutations and the nucleotide sequence of each has been determined. Each of these cloned genes will be used to overexpress or disrupt its product in order to study the purification and characterization of these proteins. It is hoped that such studies will provide insight into the mechanism of excision repair in eukaryotic cells.

Errol C.

The Journal of Cellular and Molecular Biology, 1985 (publication)

Other support: U.S. PHS, Health Services and the U.S. Department of Energy

From the Department of Pathology, Stanford University Medical Center, Stanford, CA

EXPRESSION OF THE COMPLETE HUMAN T-CELL LEUKEMIA VIRUS TYPE I pX CODING SEQUENCE AS A FUNCTIONAL PROTEIN IN *ESCHERICHIA COLI*

Human T-cell leukemia virus type I (HTLV-I), a virus associated with adult T-cell leukemia, contains a long open reading frame (LOR) in the 3′ end of its genome between the *env* region and the 3′ long terminal repeat (LTR). This open reading frame encodes a 40-kDa protein, designated p40*x*, that has been implicated as a positive control element for transcription from the HTLV-I LTR and a pleiotropic known to activate trans activation. We now report the expression of the complete p40*x* coding sequence as a 40-kDa protein in *Escherichia coli*. The p40*x* protein produced in bacteria is shown, using the protoplast fusion technique, to possess biological activity by its ability to trans-activate a HTLV-I LTR–chloramphenicol acetyltransferase plasmid that is stably integrated into the genome of mouse L cells. This stimulatory activity could be detected within 2 hr after fusion, suggesting the possibility of a direct role for p40*x* in trans-activation of the HTLV-I LTR. The production of p40*x* in large quantities in *E. coli*, together with the rapid protoplast fusion assay for its biological activity, should facilitate the analysis of p40*x* mutants and the elucidation of the molecular mechanism of trans-activation.

Gitzes, C. Z. et al.

Proceedings of the National Academy of Science, USA Vol. 83, pp. 7992–7996, October 1986.

From the Laboratory of Molecular Virology, National Cancer Institute, Bethesda, MD.

PROTEIN KINASE C PHOSPHORYLATION AT Thr 654 OF THE UNOCCUPIED EGF RECEPTOR AND EGF BINDING REGULATE FUNCTIONAL RECEPTOR BY INDEPENDENT MECHANISMS

To test the functional consequence of phosphorylation of the EGF receptor at Thr 654 by protein kinase C, the normal Thr 654 human EGF receptor cDNA or a mutant encoding an Ala 654 was expressed in heterologous cells. In cell lines expressing both the Thr 654 and Ala 654 receptors, function of cell-surface Thr 654 receptors were reduced or were totally lost, but were not degraded, following activation of protein kinase C by phorbol esters (TPA), whereas Ala 654 receptors were unaffected. These data suggest that protein kinase C regulates ligand-independent receptor binding and internalization via phosphorylation of Thr 654 of the EGF holoreceptor. Because EGF induces internalization and degradation of the Ala 654 EGF receptor, at least two independent mechanisms can serve to signal loss of functional EGF receptors.

Ito, C. J., Chen, W. S., Lazar, C. S., Carpenter, G. D., Gill, G. N., Evans, R. M., and Rosenfeld, M. G.

Cell **44**, 839–848, 1986.

Other support: National Institutes of Health and the American Cancer Society.

From the Eukaryotic Regulatory Biology Program and Department of Chemistry, University of California, San Diego, La Jolla; Molecular Biology and Virology Laboratory, The Salk Institute, La Jolla; and the Howard Hughes Medical Institute, La Jolla.

THE EGF RECEPTOR: STRUCTURE, REGULATION AND POTENTIAL ROLE IN MALIGNANCY

Retroviral *onc* genes are derived from cellular proto-oncogenes that may function in normal cellular growth control. The epidermal growth factor/EGF receptor is the proto-oncogene of *erbB*, both possess intrinsic protein tyrosine kinase activity, a property shared by several retroviral *onc* genes. The EGF receptor is a transmembrane glycoprotein with an external EGF binding domain and a cytoplasmic region that is homologous with other tyrosine kinases. *erbB* lacks the EGF binding and carboxyl terminal regions, which are thought to be important in regulation. The EGF receptor is regulated by several mechanisms: stimulation by ligand binding and self-phosphorylation; inhibition by heterologous phosphorylation and down-regulation by ligand. EGF binding stimulates several early events, including phosphatidylinositol (PI) turnover in A431 cells. A PI kinase activity co-purifies with the EGF receptor and some other tyrosine kinases. Its function is a contaminant as it can be separated from the EGF receptor. Although the role of proto-*onc* genes in human malignancy is incompletely defined, increased numbers of EGF receptors are present in several types of human tumors. Overexpression of EGF receptors, as occurs in human epidermoid carcinoma A431 cells, can augment cell growth because of increased formation of active ligand receptor complexes. Gene amplification is the mechanism underlying overexpression of EGF receptors in A431 cells and in some glioblastoma multiforme tumors.

Thompson, D. M. and Gill, G. N.

Cancer Surveys **4**: 767–788, 1985.

Other support: National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and the American Cancer Society.

From the Department of Medicine, University of California, San Diego, School of Medicine, La Jolla.

EFFECTS OF EPIDERMAL GROWTH FACTOR RECEPTOR CONCENTRATION ON TUMORIGENICITY OF A431 CELLS IN NUDE MICE

To test the relationship between the concentration of epidermal growth factor (EGF) receptors and tumor growth *in vivo*, we measured the rate of growth of several independently isolated A431 cell lines in athymic mice. This series of A431 clonal variants with differing extents of EGF receptor gene amplification and protein expression were implanted into athymic mice and the time to solid tumor formation and rate of growth were measured. Results of these experiments indicate that the degree of gene amplification and concentration of EGF receptors are directly correlated with the growth of these cells as solid tumors in host animals. Complementary DNA/hybridization analysis revealed no change in the extent of gene amplification and expression in implanted cells *versus* excised tumors or any evidence of further gene rearrangement *in vivo*. Although concentration of EGF receptors appears to facilitate the growth of tumor cells *in vivo*, it is not

Supported by U.S. Public Health Service Grant CA-[illegible] from the National Cancer Institute.

Cancer Research 47, 4647–4654, September 1, 1987

Other support: American Cancer Society and the National Institutes of Health.

From the Department of Medicine, University of California, San Diego, School of Medicine, La Jolla.

CHRONIC INHALATION STUDIES IN MICE. II. EFFECTS OF LONG-TERM EXPOSURE TO 2R1 CIGARETTE SMOKE ON C57BL/6 Cum × C3H AnCum F1 MICE[1]

Standardized exposure conditions with Kentucky reference 2R1 cigarettes were used to expose 2,088 C57BL/6 Cum × C3H AnCum F1 female mice to undiluted fresh whole cigarette smoke. In addition, 1,084 mice were sham-exposed, and 444 mice were held as cage controls. The protocol entailed exposing mice to smoke or sham exposure on a daily basis, 5 days/week, for 110 weeks and observing remaining mice until death. A large number of animals was used so that the smoke generation and animal holding systems could be tested and evaluated and yet provide significant numbers of animals for exposure to cigarette smoke for a major portion of their lifetime. Deposition of smoke particulates was estimated to be about 125–200 μg total particulate matter/g body weight/day. The only lung cancers observed were diagnosed as adenocarcinomas (AAC). A total of 39 of 978 smoke-exposed mice and 2 of 651 sham-exposed mice were observed with AAC. The difference between the smoke- and sham-exposed groups was not statistically significant at $P = .05$. But the data suggested that the tumors occurred with a shorter latency in the smoke-exposed group ($P = .10$). The data were analyzed by various methods, including analysis of subsets of the population of animals. A significant increase in the incidence of lung cancer was observed in one subset; however, this difference was not found in the population as a whole or as a result of any other analyses. Under these exposure conditions, 2R1 cigarette smoke would seem to have weak carcinogenic activity in mouse lung. Other changes associated with smoke exposure were increased incidence of pigmented alveolar macrophage accumulation, otitis media, and head and neck tissue lesions. However, the incidence of hematopoietic cancers (e.g., leukemias, lymphosarcomas, reticulum cell sarcomas), and pulmonary congestion was significantly higher in the sham-exposed animals.

From the Chemical Research, Development and Technology Department, Philip Morris Research Center, Richmond, VA 23261

Journal of the National Cancer Institute, 77:413–434, 1986.

Reprint: MicroPublishers Associates, Bethesda, MD.

ENHANCED *IN VITRO* SELECTIVE TOXICITY OF CHEMOTHERAPEUTIC AGENTS FOR HUMAN CANCER CELLS BASED ON A METABOLIC DEFECT

An enzymatic defect that is prevalent in human cancer cell lines was exploited to selectively kill these cells with 6-thioguanine and human fibroblasts

CANCER METABOLISM AND TRANSMETHYLATION

[illegible] nature of cancer is outlined and it is concluded that many changes in the cellular phenotype are required for clinical cancer to occur in humans. These large [illegible] genetic changes are not as compatible with mutation theories as they are with altered methylation, which can and imbalanced transmethylation including DNA hypomethylation which are prevalent in all surveys of human cancer. Transmethylation is affected by [illegible] carcinogens, further supporting the authors' hypothesis. Applied [illegible] affected methionine metabolism, transmethylation, for cancer prevention and [illegible] are discussed.

Investigators: E. M. [illegible] and Stern, P. H.

Publication: *Modulation and Drug Design*. The Humana Press, 215-225, 1984.

Other support: National Cancer Institute, the George A. Jacobs Memorial Fund for Cancer Research, and the Dr. Louis Sklarow Memorial Fund.

From the Department of Pediatrics, University of California, San Diego, La Jolla.

MECHANISM OF REPLICATION OF ULTRAVIOLET-IRRADIATED SINGLE-STRANDED DNA BY DNA POLYMERASE III HOLOENZYME OF *ESCHERICHIA COLI*

Replication of UV-irradiated oligodeoxynucleotide-primed single-stranded ΦX174 DNA with *Escherichia coli* DNA polymerase III holoenzyme in the presence of single-stranded DNA-binding protein was investigated. The extent of initiation of replication on the primed single-stranded DNA was not altered by the presence of UV-induced lesions in the DNA. The elongation step exhibited similar kinetics when either unirradiated or UV-irradiated templates were used. Inhibition of the 3′→5′ proofreading exonuclease activity of the polymerase by dGMP or by a *mutD* mutation did not increase bypass of pyrimidine photodimers, and neither did purified RecA protein. In contrast, single-stranded DNA-binding protein stimulated bypass since it also [illegible] the rate of full-length DNA decreased 5-fold. Termination of replication at [illegible] by a process involved dissociation of the polymerase from the DNA, [illegible] could then reinitiate replication at other available primer templates. Based on these observations, a model for SOS-induced UV mutagenesis is proposed.

Journal: *The Journal of Biological Chemistry*, **261**:9528-9533, July 15, 1986.

From the Department of Biochemistry, The Weizmann Institute of Science, Rehovot, Israel.

REPLICATION OF UV-IRRADIATED SINGLE-STRANDED DNA BY RNA POLYMERASE-DEFICIENT HOLOENZYME OF *ESCHERICHIA COLI*: EVIDENCE FOR BYPASS OF PYRIMIDINE PHOTODIMERS

Replication of UV-irradiated circular single-stranded phage M13 DNA by *Escherichia coli* DNA polymerase III (EC 2.7.7.6) and DNA polymerase III holoenzyme

(E.C. 2.7.7.7) in the presence of single-stranded DNA templates that contain [illegible] lesions was partially replicated products were [illegible] with [illegible] G DNA polymerase I (E. coli DNA polymerase I) or T4 DNA polymerase with a synthetic oligonucleotide. The fraction of full-length DNA was several orders of magnitude lower than the products of pyrimidine photodimers were not absolute blocks to DNA replication. Recent models have suggested that pyrimidine photodimers are absolute blocks to DNA replication and that SOS-induced proteins are required for their bypass. Our results demonstrate that under in vitro replication conditions E. coli DNA polymerase III holoenzyme can insert nucleotides opposite pyrimidine dimers significantly even in the absence of SOS-induced proteins.

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Proceedings of the National Academy of Sciences of the U.S.A. 83 4556-4560 Pub. July 1986

From the Department of Biochemistry, The Weizmann Institute of Science, Rehovot, Israel.

DNA METHYLATION AND HEPATOCARCINOGENESIS: PERSISTENCE OF ONE-CARBON UNITS TO DIET

Groups of male Fischer 344 rats were fed either a diet supplemented with choline, folic acid, and vitamin B12 or a basal diet lacking these nutrients. Rats were killed at intervals during 14 months. Hepatic DNA was isolated and digested with restriction endonucleases. Digests were fractionated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with a nick-translated, 32P-labeled probe for albumin DNA sequences. Hybridization patterns revealed a progressive loss of the albumin gene from the livers of rats fed the basal diet. Large hepatic nodules or carcinomas were found in 11 of 12 rats fed the basal diet for 14 months. DNA was prepared from the livers of rats fed the basal diet for 14 months, and the albumin gene was analyzed by restriction endonuclease digestion and Southern blotting. DNA was found to be deficient in the albumin gene in the livers of rats fed the basal diet for 14 months, which is the time at which hepatic nodules or carcinomas were found. In addition, the albumin gene was found to be deficient in DNA from hepatic nodules or carcinomas. Therefore, a deficiency in one-carbon units in the diet may be a factor in the development of hepatic neoplasia.

Cancer Research

Cancer Research (Philadelphia) 1

Other issues of Cancer Research

Biochemical Department, Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London SW3 6JB.

BREAST CANCER GENETICS

Several [illegible] that [illegible] of the total [illegible] breast cancer. [illegible] Registry [illegible] of breast cancer had been [illegible] the end of this century.

These investigators have emphasized the distinctive natural history of [illegible] breast cancer, which is characterized by an earlier age of onset, greater bilateral[illegible] vertical transmission consistent with an autosomal dominantly inherited factor, heterogeneous tumor associations, and improved survival when compared to sporadic counterpart. Hereditary breast cancer appears to be autosomal dominantly inherited, as all of the heterogeneous variants which we have investigated. However, the development of cancer prone genes located on one of the autosomal chromosomes as opposed to the X or Y chromosomes.

Lynch, H. T. and Lynch, J. F.

In: Muller, Weber (eds.): *Familial Cancer*. First International Research Conference, Basel 1985, pp. 20–24 (Karger, Basel 1985).

From the Department of Preventive Medicine/Public Health, Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha, NE

GENETICS AND COLORECTAL CANCER

Knowledge of hereditary colon cancer syndrome diagnosis can be used for preventive patient benefit through identification of those patients with [illegible] known risk (>50%) risk for specific forms of cancer, depending upon the syndrome of concern. Surveillance and management strategies must be based upon the [illegible] in the natural history of hereditary colonic cancer, with particular attention given to problems of phenotypic variation and genetic heterogeneity.

Lynch, H. T. and Lynch, J. F.

In: Muller, Weber (eds.): *Familial Cancer*. First International Research Conference, Basel 1985, pp. 72–77 (Karger, Basel 1985).

From the Department of Preventive Medicine/Public Health, Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha, NE

FAMM[illegible] SYNDROME: FAMILIAL MULTIPLE MOLE MELANOMA SYNDROME

Within the past decade, a new cancer associated genodermatosis has been discovered: the familial atypical multiple mole melanoma (FAMMM) syndrome. This dominantly inherited trait, with extant heterogeneity and variable expressivity, has clinical and histopathologic findings exemplified by its name. The extent of patients with atypical moles in the U.S. population has been estimated to be between 2 and 8% of Caucasians. Some individuals will have a lifetime risk for malignant melanoma (MM) which may be greater than 50%. In the U.S. during 1985, there will be approximately 22,000 new cases of MM. Thus, there may be more than 1 FAMMM genotype with determining propensities for MM to account for the discrepancy in the incidence of MM.

Both the clinical and histopathologic definitions of the FAMMM syndrome [illegible] Pearl occurs in the patient who has the florid clinical expression of the [illegible] familial atypical mole/cutaneous malignant melanoma (FAM/CMM) syndrome.

melanocytic atypia with variable dermal fibrosis and lymphocytic infiltration, thus confirming the diagnosis.

Surveillance protocols for FAMMM patients and their affected relatives must not only include cutaneous inspection monthly by the patient and semiannually by the physician, but also periodic diagnostic techniques targeted for specific cancers in the other organ systems involved in the FAMMM syndrome.

Fusaro R. M. and Lynch H. T.

In: Muller, Wieben (eds.): *Familial Cancer*. First International Research Conference, Basel 1985, pp. 118–124. Karger, Basel 1985.

Other support: The Dermatology Development Fund, Department of Internal Medicine, University of Nebraska Medical Center, Omaha.

From the Department of Internal Medicine, University of Nebraska Medical Center and the Department of Preventive Medicine, Public Health and Department of Dermatology, Creighton University School of Medicine and Hereditary Cancer Institute, Omaha.

CLINICAL IMPORTANCE OF FAMILIAL CANCER

Familial cancer comprises a significant fraction of the overall cancer burden. Malignant transformation is very complex, so much about what, in fact, is of cancer family [1]. It emphasizes the fact that cancer is common and 1 in 4 Americans will be affected during their lifetime [2]. If old men live long enough, they will develop cancer. Hence, by chance, there will be significant occurrences of familial aggregation of cancer. For example, in a study in progress of almost 2,000 consecutively ascertained cancer probands of a single oncology clinic, Lynch et al. found that 10% are compatible with hereditary cancer and 2–3% show familial clustering (≥2 or more first-degree relatives affected with cancer of the same site). Lynch has provided the following guidelines of hereditary cancer: (1) early age at onset, (2) multiple primary cancer excess and patterns of tumor sites integral to specific hereditary cancer syndromes, (3) presence of clinical signs and/or biomarkers in certain hereditary cancer syndromes, (4) predictable pattern of genetic transmission, (5) distinguishing aspects of "natural" history, and (6) improved survival in certain hereditary forms of cancer (e.g., colon malignant melanoma) when compared to their sporadic counterparts. These criteria are useful in the clinical management of cancer family and in recognition of high-risk relatives. In conclusion, cancer genetics is a rapidly evolving discipline which holds a powerful potential for modifying characteristics of selected epidemiologic and cancer genesis.

Lynch H. T., Lynch J. F. and Fusaro R. M.

In: Muller, Wieben (eds.): *Familial Cancer*. First International Research Conference, Basel, 1985, pp. 6–12. Karger, Basel 1985.

From the Department of Preventive Medicine, Public Health and Department of Dermatology, Creighton University School of Medicine and the Hereditary Cancer Institute, Omaha, NE.

GENETICS AND SMOKING-ASSOCIATED CANCERS: A STUDY OF 455 FAMILIES

Cancer risk was evaluated in relatives of 254 consecutively ascertained probands with histologically verified lung cancer, and 231 probands with other smoking-related cancers. Findings disclosed a lack of any strong evidence for increased risk in lung cancer *per se* when only lung cancer in relatives was considered. Confounding factors, most prominent of which were the effect of cigarette smoking, variations of secular trends, and the heritability of the smoking phenotype itself, tended to obfuscate identification of an inherited effect presenting itself exclusively as lung cancer liability. On the other hand, a significant increase was observed on cancers of *all* anatomic sites among the relatives of lung cancer probands ($P < 0.001$). Most of these neoplastic lesions were not associated with smoking and were not greatly influenced by secular trends. Furthermore, no significant excesses of cancer at *all* anatomic sites in relatives of probands with other smoking-associated carcinomas were observed. Thus, it may be concluded that the observation of increased risk for cancer at all anatomic sites in relatives of lung cancer probands may be a reflection of an underlying susceptibility to malignancy in these families.

Lynch, H. T. et al.

CANCER 57(8):1643-1646, 1986.

From the Creighton University School of Medicine, Boys Town National Institute for Hearing and Speech Disorders in Children, and the Hereditary Cancer Institute, Omaha, NE; and the University of Texas Medical Branch, Galveston.

FAMILIAL HETEROGENEITY OF COLON CANCER RISK

The authors have assembled family histories of cancer in 857 cancer probands, of whom 180 manifested colorectal carcinoma. This study determines if some families had a greater risk for colorectal cancer than others, and if so, what factors were associated with an increase in risk. To test for the possibility of heterogeneity of risk, a parameter called the Z-score was calculated for each family. The Z-score is a measure of the number of cancer cases in the family adjusted for the number of expected cases. A permutation test was employed to test whether or not the variance of Z-scores from the sample was greater than expected by random chance. The variance for families ascertained through colon cancer probands, but not in any of the other groups, was significantly increased. Of the colon group, 10.6% fell into a high-risk category, as did 5.56% of the rectal cancer families, but only 3.95% of the other groups combined were at high risk. Anatomic sites in the probands with the highest Z-score variances were sigmoid and transverse colon, whereas lower variances were seen for cecum and descending colon. Risk status therefore may be partially dependent upon exact anatomic sites within the colon. The effect of proband's age of diagnosis was not significant, but did show the possibility of an effect on heterogeneity of risk for both the younger and older groups.

Lynch, H. T. et al.

CANCER 57(10):2089-2096, 1986.

From the [illegible] of Preventive Medicine/Public Health, and [illegible] Department of Medicine, Creighton University School of Medicine; Boys Town National Institute for Hearing and Speech in Children; St. Joseph Hospital Oncology and Radiation Therapy Centers; and the Hereditary Cancer Institute, Omaha, NE.

BREAST CANCER GENETICS IN AN ONCOLOGY CLINIC: 328 CONSECUTIVE PATIENTS

We have provided intensive medical-genetic follow-up on a previously published cohort of 225 consecutively ascertained patients with verified breast cancer evaluated in our oncology clinic. Similar evaluation was performed on 103 newly ascertained breast cancer patients, giving a total of 328 consecutive patients with verified breast cancer. We believe that this represents one of the most intensive efforts of its type for meticulous documentation of genealogy and cancer of all anatomic sites on a series of breast cancer patients. The results showed that the familial and putative hereditary categories almost doubled among our original 225 breast cancer probands. We conclude that the impact of the host factor component in breast cancer etiology, as reflected in most reports in the literature, suffers from severe underestimation.

Lynch, H. T. and Lynch, J. F.

Cancer Genet Cytogenet **22**: 369-371, 1986

From the [illegible] Department of Preventive Medicine/Public Health, Creighton University School of Medicine, Omaha, NE.

^{31}P NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC INVESTIGATION OF HUMAN NEUROBLASTOMA *IN SITU*

Neuroblastoma is a unique tumor of childhood that has a wide range of malignant expression. The prognoses range from excellent, with minimal treatment required, for patients with localized tumors or a special pattern of widespread disease to very poor for those with skeletal metastases. Certain infants with disease classified as Stage IV-S have a different prognosis from those with Stage IV disease who have skeletal metastases or distant lymph-node involvement. Biological differences between Stages IV and IV-S may help to explain the different malignant potential of the two tumor types. The study reported here is a further attempt to understand the biology of neuroblastoma. Specifically, phosphorus nuclear magnetic resonance (^{31}P NMR) spectroscopy has recently been shown to have great potential for monitoring the metabolism of mobile phosphorylated compounds in vivo. The purpose of the study reported here was to determine whether ^{31}P NMR can (1) detect a diagnosed neuroblastoma within the body, (2) demonstrate a difference between the spectrum for "benign" neuroblastoma (Stage IV-S) and that for "malignant" neuroblastoma (Stage IV), and (3) monitor changes in tumor metabolism in response to treatment. The two infants chosen for study had enlarged livers due to neuroblastoma, which provided a unique opportunity to study [illegible] of gross hepatic involvement. The authors believe that this study was partially successful in achieving all three objectives stated above. In summary, it is concluded that ^{31}P NMR can be used to detect tumors in vivo and monitor their response to

treatment. Furthermore, the authors suggest that the phosphomonoester ATP, rather than its ester, is the effector of growth or regression of neuroblastoma and possibly other tumors.

Mares, J. M., Evans, A. E., McLaughlin, A. C., D'Angio, G. J., Bolinger, L., Manos, H., and Chance, B.

The New England Journal of Medicine **312**: 1505–1510, 1985.

Other support: National Institutes of Health, U. S. Department of Energy, Ben Franklin Partnership, the Advanced Technology Center of Southeastern Pennsylvania, and the Ronald McDonald's Children's Charities.

From the Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, and the Children's Cancer Research Center, Philadelphia.

MAPPING THYROTROPIN β SUBUNIT GENE IN MAN AND MOUSE

The hormone TSH is composed of two subunits, α and β. Previously, we have mapped the TSHα gene to human chromosome 6 and mouse chromosome 4. In this study we have located the human TSHβ gene on chromosome 1 and the mouse TSHβ gene to chromosome 3. These data suggest that the TSHβ gene lies in a conserved linkage group with the genes for amylase 1 and 2, nerve growth factor, and the proto-oncogene N-ras.

Nature (London)

Somatic Cell and Molecular Genetics **12**: 307–311, 1986.

Other support: Howard Hughes Medical Institute, National Institutes of Health, and the March of Dimes.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio.

EFFECT OF LEUKOCYTE ACTIVATION ON THE FORMATION OF HETEROTYPIC TUMOR CELL AGGREGATES *IN VITRO*

Washed human leukocytes (2×10^6 cells/ml) incubated at 37°C in a stirred cuvette with rat peritoneal mast cells (600×10^3 cells/ml) with or without the synthetic leukocyte chemoattractant fMLP (2×10^{-8} M) or biologically active concentrations of the major endogenous leukocyte attractant, C5a. Aggregation induced by the chemoattractants was detected within 3 min by a platelet aggregometer and by studying cytocentrifuge preparations. The response was amplified in the presence of cytochalasin B (5 μg/ml). Both methods enabled differentiation of the aggregates by their morphology or by autoradiographic labeling with ^{3}H-thymidine. Although tumor cells were incorporated into the leukocyte aggregates, there was no appreciable change in the number of aggregates formed. However, tumor cells in their solitary form (10% of 3×10^5 cells/ml) and in aggregates of two or more (90%) were incorporated into leukocyte aggregates within 30

[illegible] We conclude that local tumor cells can be [illegible] when leukocytes are stimulated by [illegible]. We [illegible] that [illegible] of neutrophils might affect the [illegible] tumor cells by [illegible] into microembolic cells [illegible] damage to the pulmonary endothelium.

Orr, F. W. [illegible]

International Journal of Cancer **35** [illegible]

Other support: National Cancer Institute of Canada

From the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada.

PROMOTION OF PULMONARY METASTASIS IN MICE BY BLEOMYCIN-INDUCED ENDOTHELIAL INJURY

The passage of circulating tumor cells across the vascular wall is an important step in the evolution of cancer metastases. Since tumor cells attach [illegible] to subendothelial [illegible] at sites of endothelial injury and retraction *in vitro*, we have used an established [illegible] model of pulmonary endothelial damage to examine the effects of endothelial injury on the localization and metastasis of circulating tumor cells *in vivo*. C57BL/6 mice were given a single i.v. dose of bleomycin [illegible] or multiple i.p. injections [illegible] twice weekly for [illegible] wk. Five days after the single injection or 4 days after the last i.p. injection, [illegible] iododeoxyuridine-labelled fibrosarcoma cells or unlabelled cells were injected i.v. Two to 8 times as many labelled cells were found in the lungs of bleomycin-treated animals after 24 h. Two [illegible] wk after injecting unlabelled fibrosarcoma cells, 1.4 to 5 times more metastatic lung colonies were counted in bleomycin-treated animals than in controls. Morphometric analysis of histological [illegible] demonstrated that the percentage of [illegible] by tumor in bleomycin-treated animals was between 4 and 16 times that of controls. Analysis of bronchoalveolar lavage fluids demonstrated [illegible]-fold increases of [illegible] and leakage of previously injected [illegible]-labelled albumin, indicating increased endothelial permeability. Electron microscopic examination of lungs of bleomycin-treated mice [illegible] endothelial retraction with exposure of the underlying basement membrane. Electron microscopy of [illegible] tumor cells located by autoradiography demonstrated their attachment to exposed basal lamina. Data from these experiments [illegible] support the hypothesis that endothelial damage can facilitate the metastasis of circulating tumor cells.

Orr, F. W. [illegible]

Cancer Research **46** [illegible]

Other support: National Cancer Institute of Canada

From the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada.

10023193

PULMONARY INFLAMMATION GENERATES CHEMOTACTIC ACTIVITY FOR TUMOR CELLS AND PROMOTES LUNG METASTASIS

Previous studies have demonstrated a chemotactic factor for tumor cells in inflammatory peritoneal exudates. Because the lung is a frequent site of inflammation and of secondary tumors, we looked for tumor cell chemotactic factors in alveolar inflammatory exudates and examined the effect of inflammation on the localization and metastasis of circulating syngeneic fibrosarcoma cells. Intratracheal injection of sterile carbon suspension (0.034 μ particles in 0.1 ml sterile water) were given to C57 black mice that were killed between 6 h and 28 days later. The total number of cells recovered in bronchoalveolar lavage fluids rose from 8 × 10⁶ to 240 × 10⁶ and was maximal at 4 days. Neutrophils accounted for more than 75% of the inflammatory cells in the first week when there was a greater than a 200-fold rise in the levels of glucosaminidase in lavage fluids. Injection of water alone caused a mild inflammatory response that subsided rapidly. In Boyden chambers, the tumor cells demonstrated chemotactic responses to lavage supernatants from animals with inflamed lungs, and the magnitude of response correlated directly with the number of neutrophils ($r = 0.69$) or total exudate cells ($r = 0.947$) but not with macrophages ($r = 0.05$). Intravenous injection of 2×10^{-4} 5-iododeoxyuridine labeled tumor cells on the third to fifth day after intratracheal injection was followed after 24 h by pulmonary localization of 3 to 5 times more tumor cells in inflamed lungs than in control animals. Similarly, 7 to 21 days after injection of unlabeled tumor cells, there were 2 to 4 6 times more grossly detectable metastases in the lungs of animals with pulmonary inflammation ($p < 10^{-25}$). We conclude that acute pulmonary inflammation can result in the generation of chemotactic activity for neoplastic cells and may promote tumor metastases at that site.

Otis, J. W., Adamson, I. Y. R., and Young, L.

American Review of Respiratory Disease **131**:607–611, 1985

Other support: National Cancer Institute of Canada

From the Department of Pathology, University of Manitoba, Winnipeg, Canada

INDIVIDUAL VARIATION IN BENZO(α)PYRENE METABOLISM AND ITS ROLE IN HUMAN CANCER

In the concluding section of this benzo(α)pyrene metabolism study, this reported that optimization of lymphocyte cell culture and enzyme assay conditions led to the development of a reproducible assay for the measurement of aryl hydrocarbon hydroxylase (AHH) activity and inducibility in lymphocytes and monocytes. The same assay was adapted to investigate the metabolism of benzo(α)pyrene (BP) to the various metabolites formed by lymphocytes, monocytes and placenta. The high pressure liquid chromatography profiles of BP metabolism produced by lymphocytes, monocytes and placenta were qualitatively similar. 3-hydroxy BP was the major metabolite and BP-7,8-diol was produced in significant quantities by all the three tissues. Placental studies also revealed the formation of DNA nucleoside-BP metabolite adducts believed to be derived from BP-7,8-diol-9,10-oxides. Also, a very interesting observation was that lymphocyte AHH activity and inducibility in the human population varied with the season of the year, being high during summer and early fall seasons and low during other times. Studies on monozygotic and dizygotic twins demonstrated that lympho-

cyte AHH activity and inducibility were heritable traits and that AHH induction is regulated by a few genes, possibly by one or at the most two. In addition, other investigators applied the [illegible] model demonstrated "assignments" of "mutant" responses to relationship between AHH activity and the number of cigarettes smoked, although considerable interindividual variability in AHH activity was observed among maternal smokers with similar smoking histories. These investigations suggested that genetic factors are responsible for a major fraction of interindividual variability that is independent of the dose-response effects.

Gurtoo, H. L., *Paigen, B.*, and Minowada, J.

In de Serres, F. J., and Pero, R. W. (eds.): *Individual Susceptibility to Genotoxic Agents in the Human Population*, New York: Plenum Publishing Corporation, 1984., pp. 373-415.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Department of Molecular Biology, and the Department of Immunology, Roswell Park, Memorial Institute, New York State Department of Health, Buffalo.

URINARY GLUCURONIDASE AND ARYLSULFATASES IN IDENTICAL TWINS OF BLADDER CANCER PATIENTS

Studies showing that bladder cancer patients have unusually high levels of urinary β-glucoronidase and arylsulfatases A and B led to the suggestion that these urinary enzymes may participate in bladder cancer etiology. An alternative explanation of the high levels of these urinary enzymes in bladder cancer patients is that the disease itself causes the elevation. Since the levels of these enzymes are genetically determined, measuring the enzymes in healthy identical twins of bladder cancer patients can test whether high levels occurred prior to bladder cancer. Five healthy, identical cotwins of bladder cancer patients, together with matched controls, were measured for urinary β-glucuronidase, arylsulfatases A and B, and two other lysosomal enzymes as controls, α- and β-galactosidases. The mean levels of all five enzymes were not very different in the cotwins and controls, suggesting that high levels of urinary enzymes observed in bladder cancer patients are a consequence of disease rather than occurring prior to disease and contributing to its etiology.

Paigen, B., Yarfitz, S., and Tabron, D.

Cancer Research **44**:3624-3626, 1984.

From the Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, NY.

ROLE OF URINARY β-GLUCURONIDASE IN HUMAN BLADDER CANCER

It is suggested that high levels of urinary β-glucoronidase may increase an individual's risk of bladder cancer by releasing free carcinogens from their inactive glucuronide conjugates in the bladder. The hypothesis derives in part from the high levels

of urinary β-glucuronidase observed in bladder cancer patients. Because some of the rat studies were based on levels of urinary β-glucuronidase and other lysosomal enzymes in the normal population as genetically determined, one would expect that if high glucuronidase levels were a predisposing factor, bladder cancer patients would have had this trait in their progeny. It was found that levels of β-glucuronidase and three other "lysosomal" enzymes, α-galactosidase, β-galactosidase, and β-hexosaminidase, were not significantly elevated in the urine of 34 progeny of bladder cancer patients compared to 34 matched controls. Additionally, 15 bladder cancer patients judged to be disease-free for a medium time of 5 years did not have elevated levels of urinary β-glucuronide when compared to a normal population of 125 individuals. Thus, the high β-levels of glucuronidase observed in bladder cancer patients are most likely a consequence of disease rather than a cause.

Burger, K., Peters, J., and Parker, R.

Cancer Research, **44**: 3629–3632, 1984.

From the Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, NY.

BASIC CONCEPTS OF THE RESISTANCE OF CARTILAGE TO TUMOR INVASION

In this review the following basic assumptions have been made. 1) Tumor cells use a variety of matrix degrading enzymes in the process of invasion into the surrounding tissues and 2) the resistance of cartilage to invasion is probably due to specific molecules which inhibit regulate matrix degrading enzymes. Investigation of the local interactions of invasion may well yield information that is basic to an understanding of tumor cell behavior and potentially useful in future cancer therapy.

Kuettner, K. E., and Pauli, B. U.

In Uhlenbruck, H. K. (ed.), *Current Concepts of Diagnosis and Treatment of Bone and Soft Tissue Tumors*, Berlin Heidelberg: Springer-Verlag, 1984, pp. 62–65.

Other support: National Institutes of Health.

From the Department of Pathology, Rush Presbyterian St. Luke's Medical Center, Chicago.

CELLULAR CHANGES IN RAT URINARY BLADDER CARCINOMAS INDUCED BY FANFT: A QUANTITATIVE ELECTRON MICROSCOPIC ANALYSIS

Morphometric data of normal and neoplastic urinary bladder epithelia have been collected from the Fischer rat FANFT model. Sequential measurements of volumes, surface areas and numerical densities of organelles and, where pertinent, cellular compartments, have been made during FANFT carcinogenesis, utilizing standard point and intersection counting methods. The data show that neoplastic transformation of the bladder epithelium, and progression of FANFT tumors are associated with increasingly large deviations of cell nuclei, macronucleoli, nucleoplasmic and cytoplasmic ratios, and

Publication info: S. M. [illegible] Worcester, R. S.

Publication info: 129 63[illegible]

Publication info: National Cancer Institute and National Bladder Cancer Project

Branch of Experimental Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, and St. Vincent Hospital, Worcester, MA.

THE MOLECULAR BIOLOGY OF BOVINE PAPILLOMAVIRUSES

The papillomaviruses are small DNA viruses which induce benign squamous epithelial papillomas or cutaneous or mucosal carcinomas. Members of this group, such as papilloma studies SV40 and the nucleopolyomaviruses have been studied in large part due to the fact that they can easily be propagated during the lytic cycle. But it was only with the advent of recombinant DNA technology which permitted cloning of the various papillomavirus genomes that detailed studies of the molecular and genetic organization of the papillomaviruses have been feasible. Many have provided sufficient quantities of viral DNA to begin a systematic analysis of the papillomavirus genome. Such work has provided the standard restriction viral genome maps. Such work has also led to the [illegible] OF PAPILLOMAVIRUS GENOME ORGANIZATION OF PAPILLOMAVIRUSES, FUNCTIONAL ANALYSIS OF PAPILLOMAVIRUS GENOMES, DNA CLONING OF [illegible] DNA. The experiments which [illegible] DNA [illegible]. The [illegible] and [illegible] these proteins may [illegible] SV40 Large T antigen (Large [illegible] DNA [illegible] the early [illegible] SV40 late promoter, this hopes [illegible] these cells to permit the detection and [illegible] the BPV-1 proteins involved [illegible]

[illegible] and [illegible] M. S.

[illegible] Worcester, MA [illegible] Supported in part by U.S. Public Health Service Grant [illegible] and National Bladder Cancer Project Grant [illegible]

Department of [illegible] National Cancer Institute, Bethesda, MD

DISSOCIATION OF TRANSFORMING AND TRANS-ACTIVATION FUNCTIONS FOR BOVINE PAPILLOMAVIRUS TYPE 1

It has been shown that genetic information encoded by the 3′ open reading frames (ORFs) E2, E3, E4 and E5 of bovine papillomavirus type 1 (BPV-1) is sufficient to induce cellular transformation of certain mouse cells. The product of the E2 ORF has further been shown to be responsible for the *trans*-activation of a transcriptional regulatory element located in the noncoding region (NCR) of the BPV-1 genome. To examine whether or not the E2 *trans*-activation functions encoded by the same gene that encodes the 3′ ORF viral transformation function, we have now analyzed the expression of the *trans* activation function in series of mouse C127 cell transformed by BPV-1 deletion mutants. In addition, using mutated complementary DNA clones generated by the insertion of a premature translational termination linker into different sites of a BPV-1 cDNA clone containing the 3′ ORFs intact, we demonstrate that transformation and transcriptional *trans*-activation functions can be dissociated and that they map respectively to the E5 and E2 ORFs.

Yang, Y. C., Spalholz, B. A., *Rabson, M. S.*, and Howley, P. M.

Nature **318** (6146): 575–577, 1985.

Other support: Damon Runyon-Walter Winchell Cancer Fund.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

MOLECULAR ASPECTS OF PAPILLOMAVIRUS-HOST CELL INTERACTIONS.

The bovine papillomavirus type (BPV-1) has served as a model for unraveling the molecular genetics of the papillomaviruses. BPV-1 transformation of rodent cells in tissue culture has provided a means to study the viral functions involved in latent infection of cells and in the induction of cellular proliferation functions. BPV-1 encodes two independent gene products, each of which can induce cellular transformation in susceptible rodent cells. In addition, BPV-1 contains a transcriptional regulatory element which can be transactivated by a specific BPV-1 early gene product, indicating [illegible] gene expression is normally regulated and controlled. These viral gene functions involved in cellular transformation and in the *trans*-activation of a transcription enhancer most likely have analogous counterparts in the human papillomaviruses. These viral functions, therefore, may be involved in the malignant progression of a benign papillomavirus-associated lesion to a squamous cell carcinoma.

Howley, P. M., Yang, Y. C., Spalholz, B. A., and *Rabson, M. S.*

Banbury Rep. 21: 329, *In Origins of Cervical Cancer*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1985.

Other support: Damon Runyon-Walter Winchell Cancer Fund Fellowship.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

LOCALIZED DNA ANALYSIS OF BOVINE PAPILLOMAVIRUS TYPE 1 TRANSFORMING FUNCTIONS

Bovine papillomavirus type 1 (BPV-1) or cloned BPV-1 DNA can transform susceptible rodent cells, and the viral DNA remains as a stable extrachromosomal plasmid in the transformed cells. The transforming region of the BPV-1 genome has previously been localized to a specific fragment comprising 69% of the genome which also contains the elements sufficient for extrachromosomal plasmid maintenance. To define more precisely the viral DNA sequences which are required for the cellular transformation, we have tested the ability of deletion mutants of BPV-1 DNA to morphologically transform mouse C127 cells. Cells containing the mutated DNAs have been examined for anchorage independence and tumorigenicity in nude mice. Several distinct regions of the BPV-1 genome were found to be involved in the expression of the viral transforming functions. A transcriptional regulatory region located in the non-coding region 5′ to the early open reading frames is essential for transcription of viral RNAs and transformation. A transcriptional enhancer element located 3′ to the polyadenylation site for the viral RNAs expressed in transformed cells, has previously been shown to be essential for transformation. Deletion mutants affecting the E2 open reading frame, particularly the eNH2 half, are significantly impaired in their ability to transform, suggesting that the E2 gene product is an important transforming protein of BPV-1. Mutants lacking the E4 and E5 open reading frames are still able to induce transformation but at a lowered efficiency, and the transformants have altered phenotypic properties. Mutations located 3′ to the E5 open reading frame do not significantly affect the transforming functions but result in the integration of the viral DNA into the host genome. Transformed cells containing the E2 gene product in stable plasmid maintenance mutants

Sarver, N., Rabson, M. S., Yang, Y. C., Byrne, J. C., and Howley, P. M.

Journal of Virology, 52, 377–388, 1984.

From the Laboratory of Pathology, National Cancer Institute, Bethesda, MD.

PAPILLOMAVIRUS TRANSFORMING FUNCTIONS

This [illegible] papillomavirus type 1 (BPV-1) [illegible] the [illegible] papillomavirus. BPV-1 transformation of rodent cells in tissue culture, [illegible] viral [illegible] infection of cells in the [illegible] BPV-1 has been shown [illegible] cellular transformation [illegible] BPV-1 genes appear to act synergistically to [illegible] C127 cells. Deletion mapping studies have shown that the [illegible] papillomavirus genome [illegible] tumorigenesis and [illegible] BPV-1 [illegible] indirectly to [illegible] BPV-1 [illegible] called the [illegible] transcription [illegible] RNA [illegible] products [illegible] activation [illegible] mutants [illegible]

Howley, P. M., Yang, Y.-C., Spalholz, B. A., and Kanda, M. S.

1986 Papillomaviruses. Wiley, Chichester (Ciba Foundation Symposium 120), pp. 30–52.

Other support: Damon Runyon-Walter Winchell Cancer Fund Fellowship and NRSA Fellowship Grant.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

THE E5 TRANSFORMING GENE OF BOVINE PAPILLOMAVIRUS ENCODES A SMALL, HYDROPHOBIC POLYPEPTIDE

Bovine papillomavirus contains two independent transforming genes that have been mapped to the E5 and E6 open reading frames (ORFs). The E5 transforming protein was identified by means of an antiserum against a synthetic peptide corresponding to the 20 COOH-terminal amino acids of the E5 ORF. The E5 polypeptide is the smallest viral transforming protein yet characterized. It had an apparent size of 7 kilodaltons. The transforming polypeptide is encoded entirely within the second half of the E5 ORF, and its predicted amino acid composition is very unusual: 68% of the amino acids are strongly hydrophobic and 34% are leucine. Cell fractionation studies localized this polypeptide predominantly to cellular membranes.

Schlegel, R., Wade-Glass, M., *Rabson, M. S.*, and Yang, Y.-C.

Science 233(4764): 464–467, July 25, 1986.

From the Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD.

THE NEW BIOMEDICAL TECHNOLOGY

New methods for studying the genetic information of humans in health and disease are emerging from basic science laboratories. Because these approaches are yielding fundamental insights for diagnosing and treating disease, it is important that practitioners begin to understand these methods and how they are used. Methods for genetic [illegible] recombinant DNA techniques consist of isolation, separation, propagation in bacteria or eukaryotes, and molecular hybridization of DNA. The study of RNA allows determination of gene expression. These methods are being used to understand cancer, identify hereditary illness, produce pharmaceuticals, and diagnose common medical problems, such as infectious diseases.

Sanborn, C. H.

The Western Journal of Medicine **143**: 819–824, 1985.

Other support: National Institutes of Health and the Eleanor Roosevelt Institute for Cancer Research.

From the Eleanor Roosevelt Institute for Cancer Research and the Department of Medicine, University of Colorado Health Sciences Center, Denver.

A METHOD FOR ISOLATING NICK TRANSLATED DNA BY SUBSEQUENT SEPARATION ON LOW MELTING TEMPERATURE AGAROSE

Nick translation of cloned DNA segments followed by separation on low melting temperature agarose has been used for cloning multiple radiolabeled DNA probes from a single nick translation procedure. The technique avoids polymerase inhibition of enzymatic reactions. Examples of the utility of the procedure are presented and the advantages and drawbacks are discussed.

Fisher, P. S., Fisher, J. H., et al.

Analytical Biochemistry, 1**40**: 25–27, 1984.

Other support: National Institutes of Health, American Lung Association, and Roosevelt Institute for Cancer Research.

From the Webb-Waring Institute and Department of Biochemistry, Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center and Rose Medical Center, Denver.

PURIFICATION OF HUMAN DNA CYTOSINE-5-METHYLTRANSFERASE

We have developed a facile procedure for the purification of DNA methyltransferase activity from human placenta. The procedure avoids the isolation of nuclei and the dialysis and chromatography of large volumes. A purification of 38,000-fold from the whole cell extract has been achieved. The procedure employs ion exchange, affinity, and hydrophobic interaction chromatography coupled with preparative glycerol gradient centrifugation. A protein of 120,000 daltons was found to coincide with the activity and was the major band seen in the most highly purified material after SDS gel electrophoresis. This observation, coupled with an observed sedimentation coefficient of 5.8S, suggests that the enzyme is composed of a single polypeptide chain of this molecular weight. Hemimethylated DNA was found to be the preferred substrate for the enzyme at each stage in the purification. The ratio of the activity of the purified product on hemimethylated to that on unmethylated MEA-depleted DNA was about 12 to 1. Thus, the purified product has the properties necessary for a maintenance methyltransferase. The availability of highly purified human DNA methyltransferase should facilitate the study of its structure, function, and expression in cells as well as its role in both normal and transformed cells.

Zweller, K. F., Rogers, S. G., et al.

Journal of Cellular Biochemistry, 29: 37–47, 1985.

Other support: National Institutes of Health.

From the Department of Molecular Biology, Biochemical Research Division of Eli Lilly Co., Hope, Dakota, CA.

CONCERTED DISPERSAL OF ALU AND L1 REPETITIVE SEQUENCES

The mechanisms by which transposable elements are dispersed throughout the genome are poorly understood. DNA [illegible] transposable elements

Purified bacteriophage strains do not show clear sequence specificity [illegible] those from mouse have been shown to prefer cytosine residues in human-phylated 5'CG dimers. These findings notwithstanding, Sano and Sager (1982) have reported that each of the repeating elements of bovine satellite DNA is methylated in a unique complex pattern of methylation within a given tissue, and that within each of these the 5'CCGG and 5'TCGA tetramers are preferentially modified. Experiments discussed in this report suggest that molecular mechanisms exist that produce correlated alterations in the methylation state of broadly interspersed, but repetitive DNA sequences.

Send reprint requests to Dr. Sano at the address above.

Biochemistry and Biophysics of DNA Methylation, Academic Press, 1985. Alan R. Liss, Inc., pp. 211–222.

Other support. National Institutes of General Medical Sciences.

From the Beckman Research Institute of the City of Hope, Department of Biological Sciences, Duarte, CA.

THE TUMORIGENICITY OF 5-AZACYTIDINE IN THE MALE LIVER RAT

5-Azacytidine was administered to young adult male Fischer 344 rats weekly for 52 weeks at doses that had been established as tolerated in a 90-day range-finding experiment. Several rats had multiple primary tumors. The tumors were classified for complete autocompetency in a variety of tumor types which could. These included adrenocortical and mammary fibroadenomas, thecomas of the ovary, and tumors of the testes, skin, and liver. No hepatic tumors were found in the group that was fed 5-azacytidine. Hepatocellular carcinomas were found only in the group that was exposed to hepatic tumor promoter. By contrast, a group of rats that was exposed to the same dose of 5-azacytidine but not to the hepatic tumor promoter showed no hepatic tumors. No tumors were found in the age-matched controls. These results suggest that 5-azacytidine is a broad-spectrum carcinogen in the rat, but that it is not a complete carcinogen in the liver.

Correspondence to: E. E. Reynolds, Department of Genetics, Stanford University, Stanford, CA 94305.

Received 5 [illegible] 1985; accepted [illegible]

Other support. National Institutes of General Medical Sciences; Western Medical Research Foundation; Pharmaceutical Research Foundation; Bristol-Myers Foundation.

From the Department of Medical Oncology and Anatomical Pathology, Department of Experimental Research, Beckman Research Institute of the City of Hope, Duarte, CA.

METHYLATION OF A MIDDLE REPETITIVE DNA SEQUENCE CLASS IN [illegible] AND ITS ALTERATION IN TRANSFORMED CELLS

[illegible]

cell [illegible] and stable durable differentiation induced by BMS.

Tolberg, M. E., and Smith, S. S.

FEBS Letters 176(2):280-284, October 1984.

Other support: National Institutes of Health.

From the Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, Ca.

STRUCTURAL ORGANIZATION OF INTERSPERSED REPETITIVE ELEMENTS PRESENT IN THE DNA OF *MUS MUSCULUS*

Hybridization displays of the restriction fragments from the DNA of *Mus musculus* revealed a complex species specific pattern produced from nonsatellite repetitive sequences. The patterns have been used as a guide in the direct purification of a group of broadly interspersed repeated DNA sequences (characterized by a 1350 bp *Eco*RI fragment) that have been studied by molecular cloning, restriction mapping and genomic Southern blotting. These studies show that the cloned representatives originate from an abundant group of sequences that share homology with about 2% of the mouse genome. The sequences do not appear to share homology with mouse interspersed family-1 (MIF-1) nor with the major AT-rich satellite sequences of mouse. They appear to be part of a group of larger repetitive elements that is both broadly interspersed and heavily methylated in normal mouse tissue.

Tolberg, M. E., and *Smith, S. S.*

Biochimica et Biophysica Acta **783**:272-282, 1984.

Other support: National Institutes of Health.

From the Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA.

SELECTIVE PHOSPHORYLATION OF HUMAN DNA METHYLTRANSFERASE BY PROTEIN KINASE C

Human DNA methyltransferase, the enzyme thought to be responsible for the somatic inheritance of patterns of DNA methylation, is an effective substrate for phosphorylation by protein kinase C. This provides a plausible mechanistic link between the action of tumor promoting phorbol esters, which stimulate protein kinase C, and [illegible] patterns of DNA methylation often observed in transformed cells.

DePaoli Roach, A., Roach, P. J., Zucker, K. E., and *Smith, S. S.*

FEBS Letters 197(1,2):139-145, March 1986.

Other support: National Institutes of Health.

From the Departments of Enzymatic Research and Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA.

SUPPRESSION OF TUMORIGENICITY WITH CONTINUED EXPRESSION OF THE c-Ha-*ras* ONCOGENE IN EJ/BLADDER CARCINOMA-HUMAN FIBROBLAST HYBRID CELLS

A human tumor cell line (EJ) expressing an activated c-Ha-*ras* oncogene was fused with a normal human fibroblast cell line. This fusion resulted in hybrids that behaved as transformed cells in culture but failed to form tumors in nude (athymic) mice. After repeated cell passage, two tumorigenic segregants of the hybrids arose in culture. The levels of expression of activated c-Ha-*ras* mRNA and its protein product, p21, were similar in the EJ cell line, the nontumorigenic hybrids, and the tumorigenic segregants. DNA transfections of the hybrids were performed with activated c-Ha-*ras* plasmid constructs, and transfectants expressing a 2-fold level of c-Ha-*ras* relative to the hybrid cells were found to maintain the nontumorigenic phenotype. We suggest that expression of the active c-Ha *ras* oncogene is insufficient for the malignant transformation of these human cells.

Gusset, A. G., Der, C. J., Marshall, C. J., *Stanbridge, E. J.*

Proceedings of the National Academy of Sciences, USA **83**:5209-5213, July 1986.

Other support: National Cancer Institute and the Philip and Clarisse Hay Fund.

From the Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine.

SELECTIVE TRANSFER OF INDIVIDUAL HUMAN CHROMOSOMES TO RECIPIENT CELLS

Two hypoxanthine phosphoribosyltransferase-deficient human cell lines, D98-AH-2 and HT1080 6TG, were stably transfected with pSV2 *gpt*, a plasmid containing the selectable marker *Escherichia coli* xanthine-guanine phosphoribosyl transferase (Eco *gpt*). Hypoxanthine-aminopterin-thymidine-resistant transformants arose with a frequency of ca. 10^{-6} and contained mostly single, but occasional multiple, copies of the plasmid sequences. These transformants actively express the Eco *gpt* marker. Single chromosomes from two different HT1080 *gpt* transformants and one D98 *gpt* transformant, containing the integrated plasmid sequences, were transferred via microcell-mediated chromosome transfer to hypoxanthine phosphoribosyl transferase-deficient mouse A9 cells. The transferred human chromosomes were identified as 2, 4 and 22 by using a combination of G-11 staining, G-banding, isoenzyme analysis, and *in situ* hybridization. This system is being used to create a library of interspecies microcell hybrid clones, each containing a unique single human chromosome in a mouse background. The complete library will represent the entire human karyotype.

Saxon, P. J., Srivatsan, E. S., Deupree, G. V., Sameshima, J. H., and *Stanbridge, E. J.*

Molecular and Cellular Biology, 5:1430-1436, 1985.

Other support: National Cancer Institute and National Institutes of Health.

From the Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine.

PHAGOCYTES AS CARCINOGENS: MALIGNANT TRANSFORMATION PRODUCED BY HUMAN NEUTROPHILS

In a study of the relation between chronic inflammation and cancer, a process, C3H mouse fibroblasts of the JOLLY 2 clone 8 line (JoLT 2 cells) were exposed to human neutrophils stimulated to synthesize reactive oxygen intermediates [illegible] a cell free enzymatic system generating superoxide: xanthine oxidase plus hypoxanthine. After exposure, the JOLT 2 cells were either placed in tissue culture or immediately injected into athymic nude mice. Both malignant and benign tumors developed [illegible] mice injected with treated cells, but not in those injected with control cells. Conditioned cells grown from benign tumor subsequently developed [illegible]. Malignant transformation was also observed [illegible]

Weitzman, S. A., Weinberg, A. F., Clark, E. P., and Stossel, T. P.

Science 227: 1231–1233, 1985.

Other support: American Cancer Society, National Institutes of Health, Bristol-Myers, and the Edwin Webster Foundation.

From the Pediatric Oncology Unit, Massachusetts General Hospital Cancer Center, and Department of Medicine, Harvard Medical School, Boston.

AUGMENTED EXPRESSION OF NORMAL *c-mos* IS SUFFICIENT FOR COTRANSFORMATION OF RAT EMBRYO CELLS WITH A MUTANT *ras* GENE

The authors studied the effect of altered *c-mos* structure and expression upon the ability of *ras* oncogenes to mediate the transformation of normal rat embryo cells when it was supplemented by Ela, the mutant cellular *ras* gene from EJ/T24 bladder carcinoma cells. We studied cellular *c-mos* alleles cloned from normal and tumor tissues of chicken and human origin and found that only EJ-*c-mos* (derived from a bladder carcinoma in which an activated *ras* virus long terminal repeat resides within the first *c-mos* intron) in the sense transcriptional orientation had cotransforming activity. No activity was observed with normal chicken and human *c-mos* alleles, two other "forms" of the human *c-mos* allele (T1-*c-mos* and T1-*c-mos*) and two human *c-mos* genes (HSR-*c-mos* and IDM-*c-mos*) from human promyelocytic and fibroblast cell lines. COLO 320 cells, which have *c-mos* amplified. Some of these inactive alleles had the following alterations that are frequently found in tumor-derived *c-mos*: point mutations affecting the encoded protein (T1-*c-mos*), a truncated structure with loss of the first normalizing exon (T1-*c-mos* and IDM-*c-mos*), an "processed" integrated without intron (HSR-*c-mos*), and [illegible] (T1-*c-mos*). The following experimental procedures indicated that [illegible] activity was directly related to the transcription of the alleles introduced into cells. Whether cotransfected into Rat 2 cells, EJ-*c-mos* was [illegible] than the other inactive alleles, and only a high level expression of HSR-*c-mos*, IDM-*c-mos*, or normal human or normal chicken *c-mos* [illegible] the transcriptional control of retroviral long terminal repeats or increased expression of normal human *c-mos* under the in-

fluence of a retroviral enhancer element was accompanied by cellular activation to develop. We concluded that augmented expression of even an normal c-*mos* gene is sufficient for cotransforming activity and that additional structural alterations [illegible] tumor-derived alleles are neither necessary nor sufficient for the gain of augmented embryo cell cotransforming properties.

Lee, W. H., Schwab, M., Westaway, D., and *Varmus, H. E.*

Molecular and Cellular Biology 5(12):3345-3356, 1985.

Other support: The George Hooper Research Foundation, National Institutes of Health and American Cancer Society.

From the Department of Medicine, Cancer Research Institute and Howard Hughes Medical Institute Laboratory, Department of Microbiology and Immunology, University of California, San Francisco.

A DEGLUCURONIDATION INHIBITOR REDUCES THE INDUCTION BY BENZO(a)PYRENE OF A 60-KILODALTON ONCOFETAL PROTEIN AND DNA BINDING *IN VIVO*

The objective of the present study was to evaluate the possibility of reducing carcinogenicity of benzo(a)pyrene (BP) in rats by the use of 2,5-di-O-acetyl-D-glucaro 1,4:6,3-dilactone (DAGDL). Our previous study demonstrated that DAGDL, a precursor of D-glucaro 1,4-lactone, known to be a natural inhibitor of β-glucuronidase, caused a 70% reduction in the incidence of mammary tumor induction in rats by 7,12-dimethylbenz(a)anthracene (DMBA). Coadministration of DAGDL also reduced the induction of a 60-Kd oncofetal protein shown to be associated with carcinogenesis and tumorigenesis, and markedly reduced binding of DMBA to organ DNA. In the present study, we have examined the effect of DAGDL coadministration on the induction of the oncofetal protein and its binding to organ DNA in rats. DAGDL suppressed by 25% the induction of the oncofetal protein. By a carcinogenic dose of BP, DAGDL also reduced BP binding to organ DNA, i.e. by 50-70%, depending on the organ examined. DAGDL appears to act as an anti-carcinogen depending on the organ examined. DAGDL appears to act as an anti-carcinogen by inhibiting deglucuronidation of hydroxylated metabolites of PAH, thereby increasing detoxification and carcinogenic clearance.

Walaszek, Z., Hanausek-Walaszek, M., and Webb, T. E.

In: Cooke, M. W. and Dennis, A. J. (eds.): *Polynuclear Aromatic Hydrocarbons: Ninth International Symposium on Chemistry, Characterization and Carcinogenesis*, Columbus, OH: Battelle Press, 1984, pp. 947-959.

Other support: National Cancer Institute.

From the Department of Physiological Chemistry and The Comprehensive Cancer Center, Ohio State University, Columbus.

DIETARY GLUCARATE MEDIATED REDUCTION OF SENSITIVITY OF MURINE STRAINS TO CHEMICAL CARCINOGENESIS

Serum β-glucuronidase activity is shown to differ quantitatively in the following strains of mice, listed in order of increasing activity: C3H, C57BL/6, BALB/c, DBA/2, ICR/+, SENCAR, A/He. The level of the enzyme in the murine strains is shown to correlate with the urinary excretion of 17-ketosteroids, which in turn reflects the endogenous level of androgens. Dietary calcium D-glucarate, an *in vivo* β-glucuronidase inhibitor, reduced the steady-state level of both β-glucuronidase and 17-ketosteroid excretion in the highly susceptible A/He and SENCAR strains to that of strains known to be resistant to chemical carcinogenesis. Sensitivity of the A/He strain is significantly reduced by dietary calcium glucarate, which is shown to activate DNA binding at 37°C, most probably by inhibiting β-glucuronidase.

Walaszek, Z., et al.

Cancer Letters 33:285-292, 1986.

Other support: National Cancer Institute.

From the Department of Physiological Chemistry and the Comprehensive Cancer Center, The Ohio State University, Columbus.

USE OF SHORT DNA OLIGONUCLEOTIDES FOR DETERMINATION OF DNA SEQUENCE MODIFICATIONS INDUCED BY BENZO[a]PYRENE DIOL EPOXIDE

Various organic agents that alkylate DNA are known to induce mutations in bacterial and mammalian cells. The process, nature and location of modified DNA sequences in such mutants are often difficult to ascertain. In this report, a 10 base-pair oligomer (*Bam*HI linker) is treated with (+)-*trans*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide and inserted into replicative form DNA of phage M13 by ligation at a specific restriction site. *Escherichia coli* are transfected with the recombinant DNA containing the alkylated target; progeny viral plaques are selected, and their DNAs are subjected to DNA sequence analysis at the region of oligomer insertion. For the alkylated inserts used in this study, the DNA sequence analysis of progeny viral DNA showed that nucleotide deletions were present in every clone examined. These deletions occurred primarily, but not exclusively, at G+C cluster regions, varied from 1 to 28 base pairs in length, and involved both target and nontarget nucleotides. A second type of repair, which restores most of the original nucleotide bases in the alkylated insert, is also implied by the DNA sequence data obtained.

Wei, S.-J. C., Desai, S. M., Harvey, R. G., and Weisz, S. B.

Proceedings of the National Academy of Sciences, USA 81:5949-5953, 1984.

Other support: U. S. Department of Energy, American Cancer Society and National Cancer Institute.

From the Franklin McLean Memorial Research Institute, the Department of Biochemistry and the Ben May Laboratory for Cancer Research, University of Chicago.

BACTERIA ASSOCIATED WITH OBSTRUCTIVE PULMONARY DISEASE ELABORATE EXTRACELLULAR PRODUCTS THAT STIMULATE MUCIN SECRETION BY EXPLANTS OF GUINEA PIG AIRWAYS

Cell-free filtrates from broth cultures of *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae* stimulate secretion of sulfated glycoconjugates by explants of guinea pig trachea. The stimulatory effect is not related to toxicity or damage to the respiratory mucosa, as well as could be determined by ultrastructural examination of the explants after exposure. Bacteria isolated from patients with a history of chronic obstructive lung disease (*P. aeruginosa* from cystic fibrosis, *H. influenzae* and *S. pneumoniae* from chronic bronchitis) do not demonstrate increased frequency of positive strains or greater stimulation of secretion than organisms isolated from other individuals. At least three stimulatory substances are found in cell-free filtrates of *P. aeruginosa*. They appear to be proteins of molecular weight 60,000–100,000 as determined by gel filtration. Within the crude filtrate, they are relatively stable to heat, proteolysis, and storage at 4°C and in liquid nitrogen. The stimulatory activity is not lost upon subculture of the bacteria. When isolated from the filtrate by column chromatography, they become labile to heat and trypsin. Isolated active fractions show proteolytic activity coinciding with mucin-stimulating capacity, suggesting a relationship with *Pseudomonas* proteases.

Stimulatory substances released by *S. pneumoniae* and *H. influenzae* appear to be different from those elaborated by *Pseudomonas*. They are extremely labile to heat and storage, and the capacity to stimulate secretion is lost on subculture. Preliminary gel filtration indicates the *S. pneumoniae* stimulatory substance(s) is in a molecular weight range of 100,000–300,000 daltons, while that of *H. influenzae* is between 50,000 and 200,000. The results suggest bacteria which chronically infect or colonize respiratory airways of individuals suffering from obstructive lung disease can elaborate extracellular product(s) capable of stimulating secretion of mucin. Thus, the bacteria themselves may contribute to local manifestations and, ultimately, to the pathogenesis of obstructive disease.

Adler, K. B. et al.

American Journal of Pathology **125**:501-514, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology and Medicine, College of Medicine, University of Vermont, Burlington.

PREVENTIVE THERAPY OF EMPHYSEMA: LESSONS FROM THE ELASTASE MODEL

In 1964, Gross and colleagues described the first reproducible model of pulmonary emphysema produced in experimental animals by intratracheal installation of the plant protease, papain. These and many subsequent experiments have shown that

[illegible] the early [illegible] of emphysema. Things in the microenviron[illegible] [illegible] conducive to proteolysis. In several studies, cigarette smoke exposure [illegible] to potentiate elastase-induced emphysema in rats and hamsters by [illegible] protease inhibitors, altering repair, and/or enhancing phagocytic recruitment. In the study presented here, Hacey and colleagues have extended their earlier [illegible] regard to the use of intratracheal eglin c for the prevention of lung injury when purified human neutrophil elastase is subsequently instilled intratracheally. The histopathologic sequelae of elastase injury (emphysema and secretory cell metaplasia) were examined 8 weeks after intervention. The findings were that eglin c [illegible] elastase-induced injury. In a section dealing with occurrence and importance [illegible] it is suggested that human emphysema results from a chronic [illegible] injury and disordered repair. As of today, several areas seem to [illegible] rewarding investigation. These are: Induction of emphysema by other ela[illegible]. Mechanisms and control of repair processes after elastase injury. Development of improved markers of ongoing elastolytic injury, and Examination of [illegible]

Cantor [illegible]

American [illegible] of Respiratory Disease **134**:485-487, 1986

Other support: U. S. Public Health Service

From the Department of Medicine, The Jewish Hospital at Washington University Medical Center, St. Louis, MO

SEQUENCE OF PERIVASCULAR LIQUID ACCUMULATION IN LIQUID-INFLATED DOG LUNG LOBES

The [illegible] perivascular interstitium of the lung is a potential space that expands [illegible] with the formation of large liquid cuffs. To study the time course of cuff [illegible] we inflated nine isolated dog lung lobes with liquid to total lung capacity [illegible] them in liquid N after inflation periods of 1-300 min, then photographed [illegible] of each lobe at ×3 magnification. From the photographs we measured [illegible] of cuff area to vessel area for arteries and veins of 0.05-8 mm diameter [illegible] the cuff-to-vessel area ratio attained a maximum value of 3-4, which we [illegible] vessel size. However, the first cuffs to reach maximum size were [illegible] cuffs around larger vessels [illegible] slowly. No cuffs were visible around vessels smaller than 0.1 mm diameter [illegible] cuffs had formed around 90% of all vessels larger than 0.5 mm diameter [illegible] formed around only 38% of veins and 91% of arteries of smaller diameter. We [illegible] the observed rate and pattern of cuff growth using electrical analog [illegible]. The filling pattern and model analyses suggest that liquid entered the interstitium [illegible] space site associated with arteries of 0.1-1.0 mm diameter, spread [illegible] and eventually [illegible] the lobe hilum. The estimated perivascular inter[illegible] resistance decreased [illegible] 100-fold with cuff expansion.

Conhaim, R. L. [illegible] and Staub, N. C.

Journal [illegible] 60(3):513-520, 1986

1002319231

Other support. California Research Inc., Medical Education Fund of the American Lung Association of California, and Research Evaluation and Allocation Committee of the School of Medicine, University of California, San Francisco.

From the Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco.

GROWTH RATE OF PERIVASCULAR CUFFS IN LIQUID-INFLATED DOG LUNG LOBES

In the early stages of pulmonary edema, excess liquid leaving the pulmonary exchange vessels accumulates in the peribronchovascular interstitium, where it forms large peribronchovascular cuffs. The peribronchovascular interstitium therefore acts as a reservoir to protect the air spaces from alveolar flooding. The rate of liquid accumulation and the liquid storage capacity of the cuffs determine how quickly alveolar flooding is likely to follow once edema formation has begun. To measure the rate and capacity of interstitial filling we inflated 11 isolated degassed dog lung lobes with liquid to an inflation pressure of 14 cmH$_2$O (total lung capacity) for 1–300 min, then froze the lobes in liquid N_2. We made photographs of 20 randomly selected 12 × 8 mm cross sections from each lobe and measured cuff volume from the photographs by point counting. We found that cuff volume increased from 2.2% of air-space volume after 1 min of inflation to 9.3% after 300 min. To measure the driving pressure responsible for cuff formation we used micropipettes to measure subpleural interstitial liquid pressure at the hilum of three additional lobes. With liquid inflation pressure set to 18 cmH$_2$O, interstitial pressure rose exponentially to 11.5 cmH$_2$O. Interstitial compliance, calculated from our volume and pressure measurements, equaled 0.063 ml/cmH$_2$O · g wet wt^{-1}, a value similar to that measured in air-inflated lungs. Goldberg [*Am. J. Physiol.* 239 (*Heart Circ. Physiol.* 8): H189–H198, 1980] has likened interstitial filling to the charging of a capacitor, a process that follows a mono-exponential time course. A best fit mono-exponential through our cuff volume data had a time constant of 56 min, which is three times longer than that reported previously. We conclude that the previously reported time constant measured filling of only the portion of the peribronchovascular interstitium closest to the exchange vessels.

Continued [1]

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[1] *Other support.* From the California Research and Medical Education Fund of the American Lung Association of California, and the Research Evaluation and Allocation Committee of the School of Medicine, University of California, San Francisco.

From the Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco.

ANALYSIS OF A MODEL FOR POLYMORPHONUCLEAR LEUKOCYTE-INDUCED INJURY BY TOXIN EXTRACELLULAR MATRIX

Neutrophil (PMN)-induced alveolar barrier injury by inflammatory mediators is believed to be caused by the release of PMN products that subsequently interact with components of lung parenchyma. Here

we describe the interaction between activated human polymorphonuclear leukocytes (PMN) and a reconstituted human basement membrane. Purified human PMN were stimulated with phorbol myristate acetate (PMA) and then suspended in Hanks' balanced salt solution (HBSS) containing 1% human albumin and either the chromogenic peptide BNEP or the radiolabeled peptide [^{14}C]BNEP. The stimulated PMN suspension was placed on the apical surface of the amnion. Stromal and basement membrane sides of the amnion were separately exposed to the PMN. The PMN suspension was then removed and centrifuged, and the supernatant was assayed for superoxide anion (O_2^-) and for elastase activity. Injury to the amnion was evaluated by transmission electron microscopy and by measurement of [^{14}C]peptide or [^{14}C]elastin released from the membrane matrix. Although both stimulated PMN and cell-free, non-dependent release of O_2^-, only PMA-stimulated elastase release. These effects were similar when either the stromal or the basement membrane side was exposed to PMN. PMA-stimulated cells and supernatants from PMA-stimulated cells caused solubilization of matrix fragments at different incubation times. Electron microscopy confirmed the disruption of the basement membrane of the amnion by PMA-stimulated PMN. Oxidant scavengers (SOD and catalase) did not prevent matrix degradation, and elastase inhibitor by a specific chloromethylketone inhibitor diminished EN release on both sides of the amnion by activated PMN supernatants, but only at the basement membrane side by intact PMN. We conclude that in this model, elastase rather than oxygen radicals solubilizes EN from the matrix.

Sibille, Y., Lwebuga-Mukasa, J. S., Polonski, P., and Reynolds, H. Y. *J. Clin. Invest.* 74: 1204–1214, 1984.

American Review of Respiratory Disease 134: 134–140, 1986.

Other support: National Institutes of Health.

From the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, CT.

INFLUENCE OF SPUTUM IgA AND ELASTASE ON TRACHEAL CELL BACTERIAL ADHERENCE

Bacterial adherence to respiratory epithelial cells is an important pathogenetic mechanism in airway colonization, but the influence of airway proteins on this phenomenon is largely unknown. We measured tracheal cell-bacterial binding in 16 subjects with chronic tracheostomy and related these results to measurements of sputum IgA, [illegible] and other proteins from the same subjects. Tracheal cell adherence was related directly to sputum elastase activity ($r = 0.65$, $p = 0.02$) and elastase activity, primarily neutrophil proteases, was higher in subjects colonized by *Pseudomonas aeruginosa* than in those without this finding ($p = 0.02$). Sputum levels of IgA, but not protein, were related inversely to tracheal cell adherence ($r = -0.64$, $p = 0.02$). Sputum IgA concentrations, in turn, were affected by host nutritional status and airway elastase activity. Evidence that these can degrade sputum IgA was provided by an inverse relationship between these 2 proteins ($r = -0.85$, $p = 0.02$) and by *in vitro* incubation experiments showing fragmentation of IgA by purified human neutrophil elastase. Immunoblot analysis of sputum after gel separation indicated IgA fragments similar to those generated *in vitro*. These data suggest that, once adherence is established, airway colonization, the resulting inflammatory response, and

foster microbial growth by an elastase-dependent IgA cleavage and hence ciliated tracheal cell adherence.

Niederman, M. S., Merrill, W. W., Polonski, L. M., and *Gee, J. B. L.*

American Review of Respiratory Disease 133:255-260, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, CT.

BORDETELLA PERTUSSIS TRACHEAL CYTOTOXIN: DAMAGE TO THE RESPIRATORY EPITHELIUM

Few pathogens produce as many biologically diverse toxins as *B. pertussis*, and TCT is the most recently discovered component of this arsenal. Although a structurally simple molecule, its target cell specificity is so narrow that it had completely escaped detection until assayed with appropriate model systems, cultured respiratory epithelial cells. These and other models should now make it possible to evaluate the precise mechanism by which TCT selectively destroys ciliated cells, a central pathological step in the pertussis syndrome.

Goldman, W. E.

Microbiology pp. 65-69, 1986.

Other support: From the Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis.

IMPROVED ISOLATION OF RAT LUNG ALVEOLAR TYPE II CELLS: MORE REPRESENTATIVE RECOVERY AND RETENTION OF CELL POLARITY

We have developed an important procedure for rat lung alveolar Type II (ATII) cells which yields a more representative sample of ATII cells with respect to their densities. This procedure includes an initial selection on a density gradient of approximately the complete density range of rat lung ATII cells. Subsequently, to exclude contaminating macrophages and lymphocytes from this fraction, the authors have exploited the fact that the contaminating cells have leukocyte common antigen (LC) on their surfaces, whereas the ATII cells do not. Our procedure yields 2×10^7 ATII cells per rat in a fraction which is 90% pure; the cells are immediately available for biochemical or pharmacologic analysis and represent a 90–95% recovery of the ATII cells loaded onto the density gradient. The cells retain their *in vivo* morphologic characteristics, including their polarity.

Weller, N. K. and *Karnovsky, M. J.*

American Journal of Pathology 122:92-100, 1986.

From the Department of Pathology, Harvard Medical School, Boston.

ISOLATION OF PULMONARY ALVEOLAR TYPE I CELLS FROM ADULT RATS.

The authors have developed a procedure for the isolation of alveolar Type I (ATI) cells from adult rat lung. After an initial selective enzymatic digestion of the lungs by lavage with 0.2% collagenase, 0.05% trypsin, 0.008% elastase, and 0.005% DNAse Type I, the cells which are released are separated by density gradient centrifugation, and a fraction which includes all ATI cells (density, 1.0177-1.041) is harvested. Contaminating leukocytes are excluded by specific surface adsorption, exploiting the fact that these cells have leukocyte common antigen on their surfaces, whereas ATI cells do not. Similarly, contaminating alveolar Type II (ATII) cells are removed by specific surface adsorption with the use of the lectin *Maclura pomifera* agglutinin, which binds to freshly isolated ATII cells and not to ATI cells. Our procedure yields 5×10^7 ATI cells per rat in a fraction that is at least 85-88% pure, the cells are immediately available for biochemical or pharmacologic analysis and represent a 98% recovery of the ATI cells loaded onto the density gradient. The ATI cells retain their essential *in vivo* morphologic characteristics, including their polarity.

Weller, N. K., and *Karnovsky, M. J.*

American Journal of Pathology **124** 448-456, 1986.

From the Department of Pathology, Harvard Medical School, Boston.

HUMAN ALVEOLAR LINING MATERIAL AND ANTIBACTERIAL DEFENSES.

To investigate the possible antibacterial properties of human alveolar lining material (ALM), we obtained ALM and pulmonary alveolar macrophages (PAM) by bronchoalveolar lavage of healthy nonsmokers. Alveolar lining material was isolated by centrifugation or micropore filtration; electron microscopy revealed lamellar bodies, and lipid analysis showed that 98% of the lipid fraction was phospholipid. No free fatty acids were detected. *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae* (NTHI) died spontaneously in PBS at a mean rate of log 0.75 and 0.95 in 90 min, respectively; the addition of ALM appeared to exert a slight protective effect, and at higher concentrations supported replication of NTHI. There was no difference in the uptake of the bacteria by PAM when ALM was present. Phagocytosed NTHI were killed rapidly and completely within 60 min by PAM with or without ALM. A greater proportion of *S. aureus* were killed by PAM alone than in the presence of ALM. Alveolar lining material from healthy humans thus appears to have no demonstrable effect on host defense against these bacteria. The differences between our results and those of earlier studies using ALM from rats may relate to interspecies differences in the composition of ALM.

Jonsson, S., Musher, D. M., Goree, A., and *Lawrence, E. C.*

American Review of Respiratory Disease **133** 1136-1140, 1986.

Other support: Veterans Administration and the National Institutes of Health.

From the Medical Service (Infectious and Pulmonary Disease Section), Veterans Administration Medical Center, and the Departments of Medicine, Microbiology, and Immunology, Baylor College of Medicine, Houston.

EVIDENCE AGAINST LEUKOTRIENE-MEDIATION OF PROPRANOLOL-INDUCED AIRWAY HYPERREACTIVITY TO ACETYLCHOLINE

In unanesthetized guinea pigs, propranolol treatment (0.1 mg kg^{-1} i.v.) substantially increased reactivity to intravenous acetylcholine infusion or aerosolized histamine to a comparable degree. Neither BW755c (5 mg kg^{-1} i.v.), FPL 55712 (1 mg kg^{-1} i.v.), nor piriprost (5 mg kg^{-1} i.v.) pretreatment influenced propranolol's effect on muscarinic reactivity although BW755c abolished histaminic hyperreactivity. This suggests that propranolol-induced muscarinic hyperreactivity in the guinea pig is not mediated by leukotrienes whereas histaminic hyperreactivity may be.

Macias, C.

Journal of Pharmacy and Pharmacology **38**:550–552, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

LEUKOTRIENE B$_4$ POTENTIATES AIRWAY RESPONSIVENESS *IN VIVO* AND *IN VITRO*

We studied the effects of leukotriene B$_4$ (LTB$_4$) on guinea pig airway muscle responsiveness *in vivo* and *in vitro*. Responsiveness *in vivo* was assessed by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in 5 unanesthetized, spontaneously breathing guinea pigs. We found that aerosolized LTB$_4$, in a concentration that itself had no effect on baseline SRaw, caused a substantial increase in bronchial reactivity to i.v. ACh within 8 minutes of its administration. Responsiveness *in vitro* was assessed by measuring isometric contraction of the guinea pig trachealis upon stimulation by either chemical or electrical field stimuli. These studies *in vitro* showed that a concentration of LTB$_4$ that itself did not cause contraction potentiated airway smooth muscle contraction to ACh and KCl, but not to norepinephrine. This effect of LTB$_4$ was substantially reduced by nifedipine. Our data suggests that, at sites of LTB$_4$ release, the mediator may directly potentiate the responsiveness of airway smooth muscle to bronchoconstrictors.

Depaso, J. B. and *Macias, C. G.*

Prostaglandins **31**(5):898–905, May 1986.

Other support: National Heart, Lung and Blood Institute and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

8-BROMOCYCLIC GMP ABOLISHES 5-HT-INDUCED SLOW ACTION POTENTIALS IN CANINE TRACHEALIS MUSCLE

Using intracellular microelectrodes, we investigated whether 8-bromoguanosine

3',5'-cyclic monophosphate (cGMP) influenced the electromechanical effects of tetraethylammonium ... TEA on canine tracheal smooth muscle. We found that 20 mM TEA depolarized airway smooth muscle cells from −58 ± 3 mV (mean ± S.D.) to −44 ± 2 mV and caused spontaneous action potentials (APs) to develop which were 31 ± 2 mV in amplitude. These APs, and the phasic contractions electrically coupled to them, were totally abolished in buffer containing 0.1 mM cGMP. Our findings suggest that cGMP markedly affects the channels mediating TEA-induced APs in airway smooth muscle.

Richards, I. S., *Mathias*, C., Oosterhout, J. M., and Sperelakis, N.

European Journal of Pharmacology **128**:299–302, 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Departments of Medicine, Physiology, and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH

EFFECTS OF LEUKOTRIENE D_4 AND ANTAGONISTS OF ARACHIDONIC ACID METABOLISM AND CALCIUM ENTRY ON GUINEA PIG TRACHEAL MUSCLE RESPONSIVENESS

We studied the effects of antagonists of arachidonic acid metabolism and calcium entry on the responsiveness of airway smooth muscle to acetylcholine (ACh), potassium and norepinephrine. Responsiveness in vitro was assessed by measuring isometric contraction of guinea pig tracheal rings upon chemical stimulation. We found that indomethacin potentiated the response of airway muscle to ACh and KCl, but not to norepinephrine. The indomethacin-induced potentiation observed was inhibited by both BW 755C and nitrendipine. As occurred with indomethacin pretreatment, we also found that an equicontractile concentration of leukotriene D_4 (LTD_4) potentiated the responsiveness of tracheal muscle to both ACh and KCl. Our data suggest that indomethacin or LTD_4 potentiation of guinea pig airway muscle responsiveness to certain bronchoconstrictors may be mediated, at least in part, by enhanced extracellular Ca^{++} entry.

Thorpe, J. E., *Mathias*, C. G.

Prostaglandins, Leukotrienes and Medicine **24**:269–275, 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH

INDOMETHACIN INCREASES BRONCHIAL REACTIVITY AFTER EXPOSURE TO SUBTHRESHOLD OZONE LEVELS

We investigated the effects of indomethacin on bronchial reactivity after ozone exposure. Guinea pigs (two groups of six) were treated with indomethacin (30 mg/kg IP) and studied 1 h, 6 h, and 24 h after a 2 h exposure to either 1.5 or 3.0 ppm ozone. These

animals were compared to similarly exposed groups that were untreated. Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion. Prior to ozone exposure, indomethacin had no effect on either SRaw or muscarinic reactivity. In all untreated guinea pigs (n = 12) exposed to 1.5 ppm ozone, there was no significant change in SRaw or muscarinic reactivity. In contrast, all treated animals exposed to 1.5 ppm showed a substantial increase in reactivity. Those treated animals exposed to 3.0 ppm showed significant elevations in SRaw, making interpretations of changes in their reactivity difficult. We conclude that indomethacin treatment increases bronchial reactivity in guinea pigs exposed to subthreshold ozone levels.

Murlas, C. et al.

Prostaglandins Leukotrienes and Medicine **21**:259-268, 1986.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

K^+-INDUCED ALTERATIONS IN AIRWAY MUSCLE RESPONSIVENESS TO ELECTRICAL FIELD STIMULATION

We investigated possible pre- and postsynaptic effects of K^+-induced depolarization on ferret tracheal smooth muscle (TSM) responsiveness to cholinergic stimulation. To assess electromechanical activity, cell membrane potential (E_m) and tension (T_e) were simultaneously recorded in buffer containing 6, 12, 18, or 24 mM K^+ before and after electrical field stimulation (EFS) or exogenous acetylcholine (ACh). In 6 mM K^+, E_m was -58.1 ± 1.0 mV (mean $\pm$ SE). In 12 mM K^+, E_m was depolarized to -52.3 ± 0.9 mV, basal T_e did not change, and both excitatory junctional potentials and contractile responses to EFS at short stimulus duration were larger than in 6 mM K^+. No such potentiation occurred at a higher K^+, although resting E_m and T_e increased progressively above 12 mM K^+. The sensitivity of ferret TSM to exogenous ACh appeared unaffected by K^+. To determine whether the hyperresponsiveness in 12 mM K^+ was due, in part, to augmented ACh release from intramural airway nerves, experiments were done using TSM preparations incubated with [^{3}H]choline to measure [^{3}H]ACh release at rest and during EFS. Although resting [^{3}H]ACh release increased progressively in higher K^+, release evoked by EFS was maximal in 12 mM K^+ and declined in higher concentrations. We conclude that small elevations in the extracellular K^+ concentration augment responsiveness of the airways, by increasing the release of ACh both at rest and during EFS from intramural cholinergic nerve terminals. Larger increases in K^+ appear to be inhibitory, possibly due to voltage-dependent effects that occur both pre- and postsynaptically.

Mathes, C. et al.

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Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association.

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

EFFECTS OF MUCOSAL REMOVAL ON GUINEA PIG AIRWAY SMOOTH MUSCLE RESPONSIVENESS

1. The contractile response to histamine, acetylcholine (ACh), KCl or electrical field stimulation (EFS) was examined in paired tracheal rings, one of each being denuded by mucosal rubbing, which were mounted in muscle chambers perfused with a continuously aerated physiological salt solution at 37° C.

2. Removal of the respiratory mucosa increased the sensitivity of airway muscle to ACh, histamine and EFS, but not to KCl. The hypersensitivity of denuded rings to histamine and EFS was greater than to ACh. Atropine reduced the histamine hypersensitivity observed.

3. Pretreating tissue preparations with indomethacin augmented their responsiveness to EFS, histamine and ACh.

4. Indomethacin augmentation of histamine- and EFS-induced responses was greater in preparations without epithelium.

5. We conclude that the airway mucosa may be associated with a factor that reduces airway smooth muscle responsiveness to stimulation.

Marlin C

Clinical Science (1985) 70, 575–579, 1986

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and the American Lung Association

From the Departments of Medicine, Physiology and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH

O3-INDUCED CHANGE IN BRONCHIAL REACTIVITY TO METHACHOLINE AND AIRWAY INFLAMMATION IN HUMANS

The increase in airway responsiveness induced by O3 exposure in dogs is associated with airway epithelial inflammation, as evidenced by an increase in the number of neutrophils, epithelial nuclei, lymphocytes, basophils in epithelial biopsies and in bronchoalveolar lavage fluid. We investigated in 10 healthy human subjects whether O3-induced hyperresponsiveness was similarly associated with airway inflammation by examining changes in the types of cells recovered in bronchoalveolar lavage fluid obtained after exposure to air or to O3 (0.4 or 0.6 ppm). We also measured the concentrations of cyclooxygenase and lipoxygenase metabolites of arachidonic acid in lavage fluid. We measured airway responsiveness to inhaled methacholine aerosol before and after each exposure and performed bronchoalveolar lavage 3 h later. We found more neutrophils in the lavage fluid from O3-exposed subjects, especially in those in whom O3 exposure produced an increase in airway responsiveness. We also found significant increases in the concentrations of prostaglandins E_2, I_2 and thromboxane B_2 in lavage fluid from O3-exposed subjects. These results show that in human subjects O3-induced hyperresponsiveness to methacholine is associated with an influx of neutrophils into the airways and with, but not the levels of, airway cyclooxygenase metabolites of arachidonic acid.

Seltzer, J., Bigby, B. G., Stulbarg, M., and *Nadel, J. A.*

Journal of Applied Physiology **60**(4):1321-1326, 1986.

Other support: National Heart, Lung and Blood Institute, California Air Resources Board, Fisons Corporation, Vick Divisions Research, and the National Cystic Fibrosis Foundation.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

ERYTHROCYTES FROM CIGARETTE SMOKERS CONTAIN MORE GLUTATHIONE AND CATALASE AND PROTECT ENDOTHELIAL CELLS FROM HYDROGEN PEROXIDE BETTER THAN DO ERYTHROCYTES FROM NONSMOKERS

Recent observations regarding the ability of intracellular erythrocyte (RBC) antioxidants to decrease O_2^- metabolite-mediated injury to lung tissues have prompted interest in the RBC antioxidants of patients with lung disease. We found that RBC from 14 healthy, age- and gender-matched cigarette smokers contained more ($p < 0.05$) glutathione (6.3 ± 0.4 μM g Hgb) versus 5.0 ± 0.3 μM g Hgb) and catalase (249,533 ± 8,307 units g Hgb versus 222,617 ± 7,180 units g Hgb) than did RBC from nonsmokers. In contrast, RBC from cigarette smokers and nonsmokers contained the same activities of glutathione peroxidase (21.4 ± 1.2 units g Hgb versus 20.4 ± 5.5 units g Hgb). RBC from cigarette smokers also protected bovine pulmonary artery endothelial cells in culture from hydrogen peroxide (H_2O_2) better ($p < 0.05$) than did RBC from nonsmokers (52.1 ± 6.1% protection versus 31.9 ± 5.7% protection). The results suggest that alterations in RBC antioxidants may reflect exposure and/or affect susceptibility to oxidant-induced injury.

Toth, K. M., Berger, E. M., Beehler, C. J., and *Repine, J. E.*

American Review of Respiratory Disease **134**(2):281-284, 1986.

Other support: National Institutes of Health, American Lung Association, Procter & Gamble, and Tambrands, Inc.

From the Departments of Medicine and Pediatrics and the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

PROCESSING OF ANGIOTENSIN AND OTHER PEPTIDES BY THE LUNGS

Understanding the interactions of soluble or circulating components of the renin-angiotensin system and the kallikrein-kinin system with cellular components is important because both systems function, at least in some respects, as local hormones. However, even their systemic actions may presume critically important reactions with cellular components that are not uniformly distributed within organs or within particular cell types of a given organ or tissue. One unique feature of the pulmonary circulation is the remarkable selectivity of its metabolism of these peptides. Over the past several years, aspects of the metabolism of kinins and angiotensin I by pulmonary endothelium have been defined. The focus of this author has been on pulmonary endothelial cells which selectively process a series of vasoactive substances. Studies have shown that endothelial cells of the lung can be considered a very complex solid

phase reactor capable of extremely fast reactions of high selectivity, perhaps even specificity. This chapter details the known characteristics, functions and metabolism of the bradykinin-angiotensin family of peptides in the pulmonary circulation and includes a discussion of recent advances in the study of relevant pulmonary enzymes. The utility of cultured pulmonary endothelial cells as a model system is also evaluated and certain clinical aspects of the kallikrein-kinin and renin-angiotensin systems are reviewed and discussed.

Ryan, U. S.

In: Fishman, A. P. and Fisher, A. B., eds. *Handbook of Physiology. The Respiratory System II*. American Physiological Society, 1985, pp. 351-364.

Other support. National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

METABOLIC ACTIVITY OF PULMONARY ENDOTHELIUM MODULATIONS OF STRUCTURE AND FUNCTION

Endothelial cells play a number of active roles. They act as a barrier with limited permeability, they process circulating hormones and other bioactive materials with great efficiency and selectivity, they share the responsibility for hemostatic reactions with cells and components of blood, but under normal conditions interact very little with blood cells or blood-borne clotting factors. The enzymes, enzyme inhibitors, receptors, and transport proteins responsible for the outcome of interactions of endothelial cells with circulating substances are in many cases known. Some of the active substances released by endothelial cells have also been documented, but undoubtedly many remain to be discovered. The active antithrombogeneity of endothelium is probably due in part to release of prostacyclin, but even when prostacyclin is inhibited endothelium does not become thrombogenic. In fact, only when endothelial cells in vitro are transformed do they become thrombogenic. Under these circumstances the polarity of the endothelium may be lost and the distinction between the glycocalyx on the luminal surface and the basement membrane materials on the abluminal surface may become confused. The full range of properties and functions of the normal glycocalyx are not known, but it is clear that endothelium can play an active role in the events leading to microvascular occlusion and damage. The endothelial surface over which blood normally flows unimpeded can become a focus for procoagulant and complement-linked reactions, and such changes may be accompanied by a disarray of the glycocalyx. It is tempting to speculate that the glycocalyx is important in the recognition processes between endothelial cells and blood elements and that alterations in hemostatic and immunologic potentials are accompanied by alterations in the endothelial glycocalyx.

Ryan, U. S.

Annual Review of Physiology **48**:263-277, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

RECEPTOR-MEDIATED BINDING OF C1q ON PULMONARY ENDOTHELIAL CELLS

Normal undamaged pulmonary endothelial cells appear to be immunologically privileged in that they do not express receptors for the Fc portion of IgG nor for C3b. However, these receptors become unmasked on endothelial cells injured by viral infection or exposure to white cell lysates. We now present evidence to indicate that C1q binds to specific receptors on the surface of normal healthy endothelial cells. The binding is dose-dependent, reversible and saturable. Furthermore our data show that binding of C1q to endothelial cells is via the collagenous portion of the molecule not via the globular head regions. Thus binding of C1q to endothelium would have the effect of exposing Fc receptors that could then bind to IgG of circulating immune complexes. That Fc receptors are in fact exposed is shown by rosette formation with antibody sensitized erythrocytes. With 2C1r-2C1s-associated C1q, no binding occurred using gel fixation and transfer assays. Our results indicate that C1q binding to endothelium provides a means for localizing immune complexes on pulmonary vessels and may be important in the initiation and progression of the inflammatory response.

Zhang, S. C., Schultz, D. R. and *Ryan, U. S.*

Tissue and Cell **18** (1) 13–18, 1986.

Other support: U. S. Public Health Service.

From the University of Miami School of Medicine, Department of Medicine, Miami, FL.

REACTIONS AT THE PULMONARY ENDOTHELIAL CELL SURFACE

Since 1967, it has become evident that a wide variety of biochemical reactions occur at the surface of vascular endothelium. Many of these reactions appear to occur continuously (e.g., metabolic processing of vasoactive substances) while others occur episodically (e.g., expression of procoagulant activities and interactions with complement). Certain of both types of reactions are now understood in fair detail and in terms of structure-function correlations. The text that follows focuses 1) on continuous interactions of endothelial surface peptidase enzymes with blood-borne oligopeptide hormones, and 2) on induction of procoagulant activities and complement and immune component receptors consequent to endothelial injury. The episodic activities appear related in part to disarray of the normally highly organized glycocalyx. Of the continuous activities, the most clearly understood are the interactions of endothelium with two oligopeptides, the hormone bradykinin and the prohormone angiotensin I. Angiotensin I is converted into the vasopressor hormone angiotensin II, and bradykinin, a vasodepressor hormone, is inactivated by angiotensin converting enzyme. The latter enzyme is disposed on the endothelial surface such that it has virtually free access to its blood-borne substrates. As a consequence, endothelium can be said to regulate the hormonal composition of blood moving downstream. In the case of pulmonary endothelium, downstream blood is systemic arterial blood.

Ryan, U. S. and Ryan, J. W.

Current Mechanisms of Transport of Solutes from Blood to Tissue. Longman, London, 1985, pp. 223–236.

Other supp[illegible] National Heart, Lung, and Blood Institute of the National Institutes of Health

From the [illegible] of Medicine, University of Miami School of Medicine, Miami, FL

THE ENDOTHELIAL SURFACE AND RESPONSES TO INJURY

Pulmonary microvascular endothelial cells are known to play an active role in the events that follow vascular damage in the inflammatory response. The endothelial surface is normally physically privileged and very actively antithrombogenic, can respond to certain stimuli generally injurious by becoming strongly procoagulant and by expressing C3 and C3b receptors. Like macrophages, activated endothelial cells can provide a procoagulant source and substratum for combined hemostatic and complement-linked [illegible]. Such transformations of endothelial functions may involve alterations in [illegible] of glycocalyx and, in addition, may be important for the entrapment and [illegible] of phagocytosed particulates. What is clear is that it is not simply absence of endothelium that has a bearing on the outcome of inflammatory stimuli but the [illegible] structural and functional responses of the endothelial surface to injury, resulting in altered expression of hemostatic and immunologic potential, may have an important bearing on the role of the endothelium in the regulation of microvascular permeability.

Rec[illegible]

Federation Proceedings 45 (6): 108, 1986

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL

PULMONARY ENDOTHELIAL CELL KILLING BY HUMAN NEUTROPHILS: POSSIBLE INVOLVEMENT OF HYDROXYL RADICAL

Human blood neutrophils stimulated by a variety of agents were shown to have cytotoxic effects on bovine pulmonary artery endothelial cells. Effective agonists included immune complexes, opsonized zymosan, and 12-*O*-tetradecanoyl phorbol acetate. Unstimulated human neutrophils and neutrophils stimulated with *N*-formyl methionyl leucyl phenylalanine or with platelet activating factor failed to induce significant killing even though secretory release of lysosomal enzymes occurred. In comparing the [illegible] with endothelial cell killing showed a better correlation with the production of H_2O_2 than with the generation of O_2^-. Endothelial cell killing by stimulated human neutrophils was inhibited by catalase but not by soybean trypsin inhibitor or superoxide dismutase. Killing was also inhibited by two scavengers (*N*, *N*-dimethylthiourea and D-mannitol) of hydroxyl radical and by deferoxamine mesylate, an iron chelator. Iron-saturated deferoxamine mesylate was significantly less effective in protecting the endothelial cells against killing. Agents that were protective against [illegible] cell killing did not interfere with the generation of O_2^- by stimulated [illegible]. These results suggest that leukocyte-induced endothelial cell killing is [illegible] by [illegible] but not all agonists from neutrophils and that the killing mechanism [illegible] may be directly due to hydroxyl radical production.

National [illegible] Vol. 6, [illegible] Proc. U.S.

[illegible] 53:[illegible] [illegible]

Other support: University of Michigan and the University of Miami.

[illegible] Department of Medicine, University of Miami School of Medicine, Miami, FL

DIRECT EFFECTS OF *E. COLI* ENDOTOXIN ON STRUCTURE AND PERMEABILITY OF PULMONARY ENDOTHELIAL MONOLAYERS AND THE ENDOTHELIAL LAYER OF INTIMAL EXPLANTS

The direct structural, metabolic, and physiologic effects of *Escherichia coli* endotoxin on bovine pulmonary endothelial monolayers and on the intact endothelial layer of bovine pulmonary artery intimal explants were examined. Endothelial monolayers exposed to *E. coli* endotoxin (0.001, 0.01, 0.1, 1.0, and 10 μg/ml) for 24 hours in the absence of bovine fetal calf serum (FCS) showed a dose-dependent response, as demonstrated by number of pyknotic cells and lactate dehydrogenase release that was enhanced by addition of FCS. Prostacyclin production was increased only in the presence of FCS. Endotoxin also caused an increase in permeability. Endothelial cells [illegible] seeded on filters placed in chemotaxis chambers with radioactive tracers in the upper well showed a significant 25% increase in tracer equilibration (counts in lower well [illegible] of upper well) at 1 hour after 2 and 3 hours' incubation with endotoxin [illegible] (0.89 ± 0.05 mean ± SE; no endotoxin = 0.69 ± 0.05) and a 40% increase in equilibration of ^{125}I-albumin at three hours (3 hours' endotoxin = 0.40 ± [illegible] no endotoxin = 0.27 ± 0.02). An increase in hydraulic conductance was also seen at 1 hour. Electron microscopy of the endothelial layer of intimal explants showed disruption of the intercellular junctions and cellular changes representing contraction, increased prominence of cytoplasmic filaments, nuclear crenations, and cytoplasmic projections at 5 and 60 minutes. From 2 hours evidence of cell death was found. These findings show that endotoxin causes structural and metabolic changes in pulmonary endothelial cells and that increase in permeability of the endothelial layer. This injury occurs in the absence of FCS but is enhanced by its addition.

M. [illegible] E. O. [illegible], Boston, U. S. and Brigham, K. L.

American Review of Respiratory Disease, **122**:143–151, 1986.

Other support: National Heart, Lung and Blood Institute and the Knox Foundation.

[illegible] Medicine, University of Miami School of Medicine, Miami, FL

GLUCOCORTICOID MODULATION OF PROSTACYCLIN PRODUCTION IN CULTURED BOVINE PULMONARY ENDOTHELIAL CELLS

The present study has examined the ability of glucocorticoids to modify the production of prostacyclin by endothelial cells derived from bovine pulmonary artery. Binding studies with [^{3}H]dexamethasone indicated that these cells possessed high-affinity binding sites for glucocorticoids (K_d approximately 4 nM). The cells released [illegible] measured serologically as its hydrolysis product 6-keto-prostaglandin F[illegible]. The release was strongly stimulated by a 5-min incubation with 500 nM bradykinin.

2 μM calcium ionophore A23187 or 2.5 μM arachidonic acid. Dexamethasone at 1 to 2 nM suppressed prostacyclin release in response to bradykinin and ionophore in a dose-dependent manner. The suppressive effects required 24 hr of incubation with the steroid. Maximal suppression of bradykinin-induced prostacyclin release was approximately 65% and that of ionophore-induced release was approximately 75%. Dexamethasone was not observed. Hydrocortisone at 2 to 4 nM inhibited bradykinin-induced release of prostacyclin by approximately 45%, but had no effect on ionophore-induced release. Neither glucocorticoid inhibited prostacyclin release in response to arachidonic acid. Triton X-100 extracts and conditioned media from cells treated with 2 nM dexamethasone failed to modify prostacyclin release when added to untreated cells. We conclude that glucocorticoids may act intracellularly to produce a soluble, phospholipase binding site-occupancy, inhibitors against mediated release of prostacyclin from bovine pulmonary endothelial cells. Inhibition appears to occur at the level of phospholipase A2 to arachidonic acid release. However, we have been unable to obtain direct evidence for the production of a macromolecular phospholipase inhibitor by steroid-treated cells in response to these low concentrations of steroid.

Critchley, D. J., Rose, G. A., and Ryan, J. W.

The Journal of Pharmacology and Experimental Therapeutics 233 (3): 655–659, 1985.

Other support: U. S. Public Health Service and the Danielle Phipps Memorial Cystinosis Trust.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL

SINGLE APICAL MEMBRANE ANION CHANNELS IN PRIMARY CULTURES OF CANINE RENAL PROXIMAL TUBULE CELLS

To investigate the cellular mechanisms responsible for the apical membrane Cl conductance in a tight epithelium, we used the patch-clamp technique to study single-ion channels in primary cultures of canine proximal epithelium. The cells contained an anion channel that had a single-channel conductance of approximately 29 pS at negative voltages in symmetrical 145-mM NaCl solutions. In symmetrical CsCl solutions the excised single-channel current-voltage relation was linear with conductance of 29 pS. The current-voltage relation was rectified by the presence of 2-carboxylate [illegible] quenched by [illegible] cells, [illegible] When [illegible] activated by addition of [illegible] source [illegible] Other [illegible] from the cells [illegible] by inhibitors [illegible] may be responsible [illegible]

W. [illegible]

Physiology [illegible] 430 [illegible]

Other support: [illegible]

From the [illegible] University of [illegible] Veterans Administration Medical Center, [illegible]

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CANINE AIRWAY SMOOTH MUSCLE

1. Airway smooth muscle cells from canine trachealis muscle were dispersed by treatment with collagenase and elastase. Cells were identified as smooth muscle by their binding of anti-smooth muscle myosin monoclonal antibodies and by their contraction in response to acetylcholine. 2. The patch-clamp technique was used to study single channel currents in cell-attached and isolated patches of membrane. The most common single channel current had a conductance of 266 ± 12 pS (mean ± SD, $n = 70$) in symmetrical 135 mM K solutions. 3. The reversal potential of the channel was unaltered by large chemical gradients for Cl, Na and Ca and was determined exclusively by the chemical K gradient. Thus, the channel is highly selective for K. 4. In both cell-attached and isolated patches of membrane, depolarization increased the frequency of channel opening and the duration of the open state. 5. In isolated patches of membrane, increasing [Ca] on the cytoplasmic side of the membrane from 10^{-7} to 10^{-6} M increased both the frequency of channel opening and the duration of the open state. 6. Tetraethylammonium, tetramethylammonium, or Cs (10 mM) on the cytoplasmic side of the membrane caused a voltage-dependent decrease in conductance of the open channel while having no obvious effect on channel kinetics. These blocks were completely reversible. Ba (10 mM) on the cytoplasmic side of the membrane slightly decreased inward currents and completely blocked outward currents through the channel. 7. External Ba (10 mM) caused a voltage-dependent decrease in inward current. External tetraethylammonium (TEA) (5 mM) completely blocked single channel currents.

McCann, J. D. and Welsh, M. J.

Journal of Physiology **372**:113-127, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

ADRENERGIC REGULATION OF ION TRANSPORT BY PRIMARY CULTURES OF CANINE TRACHEAL EPITHELIUM: CELLULAR ELECTROPHYSIOLOGY

We examined the effect of adrenergic agents on the [illegible] of primary cultures of canine tracheal epithelium. Both isoproterenol and epinephrine stimulated Cl^- secretion, as evidenced by an increase in transepithelial voltage and a fall in transepithelial resistance. Moreover, both agents appear to increase the conductance of apical and basolateral membranes. However, the pattern of response was different. Isoproterenol initially depolarized apical voltage (Ψ_a) and decreased the fractional resistance of the apical membrane (f_a). These changes are consistent with an initial increase in apical Cl^- conductance. In contrast, epinephrine acutely hyperpolarized Ψ_a and increased f_a, changes consistent with an initial increase in basolateral K conductance. Following the acute effect of epinephrine, Ψ_a depolarized and f_a decreased to values not significantly different from those observed with isoproterenol. The acute increase in basolateral K conductance produced by epinephrine appeared to result from stimulation of α-adrenergic receptors because it was reproduced by addition of the α agonist phenylephrine, and blocked by the α antagonist phentolamine. The ability of prazosin

but not yohimbine, to block the acute epinephrine induced increase in K permeability indicates the presence of α adrenergic receptors. The acute α adrenergic-induced increase in basolateral K conductance may be mediated by an increase in cell Ca because the response was mimicked by addition of the Ca ionophore A23187. In contrast, the response to isoproterenol was similar to that observed with addition of 8-bromo-cAMP and theophylline. These results indicate that both β and α adrenergic agents mediate their ion transport processes in canine tracheal epithelium. β adrenergic agents have their primary effect on the apical Cl conductance, probably via an increase in cAMP. α adrenergic agents exert their primary effect on the basolateral K conductance, possibly via an increase in cell Ca.

Welsh, M. J.

The Journal of Membrane Biology **91**: 121–128, 1986.

Other support: National Institutes of Health and the American Heart Association.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

MUCOSAL ADENOSINE STIMULATES CHLORIDE SECRETION IN CANINE TRACHEAL EPITHELIUM

Adenosine is a local regulator of a variety of physiological functions in many tissues and has been observed to stimulate secretion in several Cl secreting epithelia. In canine tracheal epithelium we found that adenosine stimulates Cl secretion from both the mucosal and submucosal surfaces. Addition of adenosine, or its analogue 2-chloroadenosine, to the mucosal surface potently stimulated Cl secretion with no effect on the rate of Na absorption. Stimulation resulted from an interaction of adenosine with adenosine receptors, because it was blocked by the adenosine receptor blocker, 8-phenyltheophylline. The adenosine receptor was a stimulatory receptor as judged by the rank order potency of adenosine and its analogues and by the increase in cellular adenosine 3',5'-cyclic monophosphate levels produced by 2-chloroadenosine. Adenosine also stimulated Cl secretion when it was added to the submucosal surface, although the maximal increase in secretion was less and it was much less potent. Part, but not all, of the lower potency of submucosal adenosine resulted from submucosal uptake and metabolism of the drug. The observation that mucosal 8-phenyltheophylline blocked the effect of submucosal 2-chloroadenosine, whereas submucosal 8-phenyltheophylline did not prevent a response to mucosal or submucosal 2-chloroadenosine, suggests that adenosine receptors are located on the mucosal surface. Thus, submucosal adenosine may stimulate secretion by crossing the epithelium and interacting with receptors located on the mucosal surface. Because adenosine can be released from mast cells located in the airway lumen in response to inhaled material, and because adenosine stimulated secretion from the mucosal surface, it may be a unique positive control like epithelium-to-airway level.

Bhatt, A. D., Clancy, G., and Welsh, M. J.

American Journal of Physiology, 251: C[illegible], 1986.

Other support: National Heart, Lung, and Blood Institute.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

III. *Heart and Circulation*

COMPARTMENTATION AND FUNCTIONAL MECHANISMS IN MYOCARDIAL FAILURE AND MYOCARDIAL INFARCTION

Changes in compartmentation and specific mechanisms in acute myocardial failure due to global ischemia and in regional myocardial ischemia in dog hearts are described. Ischemic failure was produced by periodic arrest of flow to supported heart preparations perfused with a fluorocarbon (FC-43). Sarcolemmal vesicles (SL) prepared from ischemic failing heart preparations exhibited diminished Ca^{++} binding and phosphorylation. TA-064, a beta-1 agonist, partially abolished the reduction in Ca^{++} binding and phosphorylation of SL vesicles. The addition of cyclic-AMP (cAMP) and of protein kinase (PK) increased phosphorylation of SL vesicles obtained from normal or failing heart preparations. Combination of cAMP and of PK had the greatest effect. In contrast to myocardial failure, myocardial infarction is known to produce a large variety of specific disturbances in intermediary cardiac metabolism. Apparently in ischemic failing heart preparations, Ca^{++} binding and phosphorylation of SL are decreased. The results with TA-064 and isoproterenol suggest that phosphorylation of SL may play a role in the positive inotropic effect of beta-1 agonists.

Key Words: [illegible]

In: Breuer, H., ed. *Myocardial and Metabolic Biochemistry*. New York: Plenum Publishing Incorporation, 1980, pp. 283-290.

Other support: The Margaret W. and Herbert Hoover, Jr. Foundation.

From the Huntington Medical Research Institutes, Pasadena, CA.

EFFECT OF 8-BROMO-CYCLIC GUANOSINE MONOPHOSPHATE (cGMP) ON CORONARY ARTERY CONSTRUCTION IN ISOLATED RABBIT HEARTS

The vasodilator 8-bromo-guanosine 3',5'-monophosphate (8-bromo-cGMP) effectively counteracts vasopressin-induced coronary artery constriction in a supported perfused working rabbit heart. In this preparation, the coronary arteries remain in contact with the beating heart. The obtuse marginal artery and portions of the left anterior descending coronary artery were deprived of endothelium. Perfusion was carried out with Krebs-Henseleit solution, oxygenated with disposable intan oxygenator. The internal diameter of large coronary arteries was determined by roller arteriography (injection of patent blue dye and [illegible] photography). The effect of vasopressin was tested without the [illegible] of 8-bromo-cGMP on cardiac performance (cardiac [illegible] systolic pressure, left ventricular end-diastolic pressure, maximum [illegible] left ventricular pressure [dP/dt]), mean aortic pressure and large coronary vessel radii. Total coronary vascular resistance was determined in later experiments. In addition, changes in coronary sinus partial pressure of carbon dioxide (P_{CO_2}) and pH were observed. Vasopressin alone caused a significant decline in coronary flow, myocardial oxygen consumption and coronary sinus pH. Cardiac performance

Saeed, M., Schmidt, J., Metz, M., and Bing, R. J.

Journal of Cardiovascular Pharmacology 8: 257–261, 1986.

Other support: Hoover Foundation and The Lindberg Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

PHOSPHODIESTERASE INHIBITION IN POSITIVE INOTROPIC THERAPY OF CONGESTIVE HEART FAILURE

Congestive heart failure, which affects approximately two million people in the United States, still carries a poor prognosis. Means of treatment available at the present time require additional options. Recently, a new group of compounds has been clinically and pharmacologically investigated. These agents, the so-called new positive inotropic agents, are not related to the glycosides or the beta-adrenergic stimulants. Common features of these compounds are a combination of positive inotropic effect and vasodilation, a useful combination in the treatment of congestive heart failure. The mechanism of action of these drugs consists of inhibition of myocardial phosphodiesterases with an increase in cyclic 3′,5′-adenosine monophosphate (cAMP). Intracellular [illegible] is increased through a series of reactions and the cardiac performance is improved. Other mechanisms which may play an additional role in the increase of the cardiac contractility are also discussed in this article. Experimental data on the effects of the phosphodiesterase inhibition CI 914 on cardiac performance and vascular beds, as obtained in our laboratory, are included in this report.

Blackburn, A., and Saeed, M. (*Biog. R. J.*)

Journal of Applied Cardiology 1: 361–383, 1986.

Other support: The Margaret W. and Herbert Hoover, Jr. Foundation.

From the Huntington Medical Research Institutes, Pasadena, CA.

MICROVASCULAR DISTRIBUTION OF CORONARY VASCULAR RESISTANCE IN BEATING LEFT VENTRICLE

To determine the distribution of resistance in the coronary vasculature, measurements of microvascular pressure and diameter were obtained with vasomotor tone maintained during coronary dilation produced by papaverine. We studied anesthetized, open-chest cats on fixed-jet ventilation synchronized to the cardiac cycle to eliminate respiratory-induced cardiac motion. The system for measuring microvascular pressure compensated for cardiac motion with stroboscopic illumination of the microvessels and a beat-pulse controlled electromechanical micromanipulator that moved a micropipette in synchrony with the heart. Pressures were measured with the servonull technique and diameters were measured via a video system. Resistance was estimated from the pressure gradient from the aorta to a particular class (size) of coronary [illegible]. During control conditions, with coronary vasomotor tone intact, mid-coronary [illegible] was 36 ± 3 mmHg (mean ± 1 SE) and was increased to 339 ± 52 during

DNA DAMAGE INDUCED BY NO AND OF MOUSE MYOCARDIUM

Gamboa, J. W.

Journal of Molecular and Cellular Cardiology, Vol. 18, [illegible]

Other support The Heart Foundation, the American Heart Association for Aging Research and the American Heart Association

From the Department of Biochemistry, University of South [illegible] College of Medicine, Melville

PARTICIPATION OF ENDOTHELIAL CELLS IN THE PROTEIN C - PROTEIN S ANTICOAGULANT PATHWAY: THE SYNTHESIS AND RELEASE OF PROTEIN S.

The protein C-protein S anticoagulant pathway is closely linked to the endothelium. In this paper the synthesis and release of the vitamin K-dependent anticoagulant factor protein S is demonstrated. Western Blotting after SDS PAGE of Triton X-100 extracts of bovine aortic endothelial cells grown in serum-free medium demonstrated the presence of protein S. A single major band was observed at [illegible] kDa which corresponded with protein S purified from plasma absent from cells treated with sodium warfarin. Metabolic labeling of endothelial cells with [^{35}S]methionine confirmed the synthesis of protein S. When a radioimmunoassay was used, endothelium was found to release 150 fmol/ 10^6 cells per 24h and contain 44 fmol/ 10^6 cells of protein S antigen. Protein S released from endothelium was functionally active and could potentiate activated protein C - mediated factor V, inactivation on the endothelial cell surface. Warfarin decreased secretion of protein S antigen by >90% and increased intracellular accumulation by almost twofold. Morphologic studies demonstrated intracellular protein S was in the Golgi complex, concentrated at the *trans* face, rough endoplasmic reticulum, lysosomes, and in vesicles at the periphery. In contrast, protein S was not found in vascular fibroblasts or smooth muscle cells. A pool of intracellular protein S could be released rapidly by the calcium ionophore A23187 (5 μM). This effect was dependent on the presence of calcium in the culture medium and could be blocked by TMB, which suggests that cytosolic calcium flux may be responsible for protein S release. These results demonstrate that endothelial cells, but not the subendothelial cells of the vessel wall, can synthesize and release protein S, which indicates a new mechanism by which the inner lining of the vessel wall can contribute to the prevention of thrombotic events.

Stern, D., Brett, J., Harris, H., and Nossel, H. *Proc. Natl. Acad. Sci. U.S.A.*

The Journal of Cell Biology 102:1971-1978, 1986.

Other support: Young Investigator Award from the Oklahoma affiliate of the American Heart Association, and the National Institutes of Health.

From: Thrombosis Research, Oklahoma Medical Research Foundation, Oklahoma City, and the Department of Pathology, College of Physicians & Surgeons, Columbia Presbyterian Medical Center, New York.

ELUCIDATION OF THE PATHOGENESIS OF THE ATHEROSCLEROTIC PROCESS: LETTING THE GENIE OUT OF THE CULTURE BOTTLE

The author acknowledges both the past and future importance of molecular and cellular approaches to atherogenesis, but maintains that true understanding of that process must increasingly depend on studies in the intact artery or at least perfused arterial segments. Cell-culture studies, for all their value and power, can only tell us what cells are capable of under specific conditions. For instance, many conclusions regarding LDL uptake, transport and degradation using culture techniques simply were not validated in *in vivo* experiments. Likewise, post secretory modifications of lipoproteins and cell surface antigens will only be fully understood in integrated systems. Examples of other important aspects of the pathogenesis of atherosclerosis that will need *in vivo* studies are the overall dynamics of lipoproteins metabolism and the significance of monocyte/macrophage entrapment in the artery wall. The role of

[illegible]

[illegible]

E. [illegible], N. B[illegible] & N[illegible], P. J[illegible] Arterioscler Thromb Vasc Biol. 1999 Jul;19(7):1852-8.

Other support: N[illegible] H[illegible]

[illegible]

MECHANISMS INVOLVED IN THE UPTAKE AND DEGRADATION OF LOW-DENSITY LIPOPROTEIN BY THE ARTERY WALL *IN VIVO*

The causative linkage between hypercholesterolemia and atherosclerosis is firmly established on [illegible] of evidence. Illustrative of the latter is the evidence from the Lipid Research Clinics intervention trial which clearly demonstrated that lowering LDL levels in hypercholesterolemic men significantly reduced their risk of death from coronary artery disease. Since the cholesterol in atherosclerotic lesions derives primarily from the cholesterol in circulating lipoproteins, it is reasonable to conclude that hypercholesterolemia is causative by virtue of the discovery that cholesterol enters the artery at a high rate. Thus, elevated LDL levels cause high rates of LDL entry into the artery wall. Studies leading to high rates of uptake by cells in tissues such as the artery wall [illegible] to high rates of uptake by cells in tissues such as the artery wall are [illegible] by the extracellular matrix material [illegible]. The overall balance which normally mediates normally operates to prevent accumulation of cholesterol and [illegible]. The endpoint is atherosclerosis. What we have done is simply to restate the lipid infiltration hypotheses of Virchow in slightly modernized contemporary form. However, the causative linkage could be quite different. High plasma concentrations of LDL have been [illegible] with varying degrees of certainty implicated as contributing to several other processes that may be involved in atherogenesis, as discussed in detail elsewhere, and there may be still others not yet appreciated. One or more of such factors might be equally relevant or even more relevant to lesion development than the rate of delivery of cholesterol to the tissue. A crucial question is whether the delivery of lipoprotein to and the accumulation of cholesterol in the artery wall is [illegible] in hypercholesterolemia. Certainly cholesterol accumulation is the hallmark of the [illegible] atherosclerotic lesion. Yet in all experimental lesions studied to [illegible] the [illegible] of causative events was triggered by [illegible] LDL levels were high during lesion development [illegible] if we look at established lesions [illegible] of the lesion. Thus it is not completely resolved whether [illegible] or [illegible] of possible causative factors in the initiation of the process. [illegible] LDL [illegible] in the artery wall [illegible] LDL [illegible] CA

[illegible]

[illegible]

Other support: N[illegible] H[illegible]

[illegible]

LIPOPROTEINS AND ATHEROGENESIS: CURRENT CONCEPTS

Atherosclerosis is undoubtedly a disorder of multiple etiology. Many risk factors are involved in addition to hyperlipoproteinemia—notably cigarette smoking and hypertension. The precise mechanisms by which these other risk factors are linked to the atherogenic process remain to be determined. In the case of lipoproteins, we have made remarkable progress in the past decade toward a well-defined hypothesis for atherogenesis. Already we see the potential for novel modes of intervention to be used to simply modify plasma levels of lipoproteins. As we begin to learn more about the specific processes within the artery wall that play a role in the atherogenic process, we should develop modes of intervention that will act on these processes. For example, if macrophage secretions are indeed responsible in part for endothelial damage, then drugs or immunologic interventions that counteract such damaging effects might be a direct approach to arresting the atherogenic process. As another example, if endothelial cell modification of LDL is involved in the atherogenic process, it might be possible to show it by appropriate use of antioxidants since the modification depends on oxidative damage to the LDL molecule. The possibility that free radicals are involved in atherosclerosis has been proposed previously, particularly by Harman, but very little direct evidence is as yet available to support the hypothesis. Further research is certainly warranted. As a final example, if the ability of the macrophage to "rid itself" of lipids could be enhanced, one might be able to defer the development of clinically significant atherosclerosis by such means. As we learn more about the macrophage events involved in lipid metabolism, we may develop ways of altering their activities in the proper direction and test the hypothesis.

Finally, the available evidence that hyperlipoproteinemia is a major risk factor in human atherosclerosis seems to be overwhelming. In addition to the large body of experimental, biochemical, epidemiologic, and intervention data, we are now beginning to develop a rational and testable set of hypotheses regarding the mechanisms linking hyperlipoproteinemia to atherogenesis.

Stan Parker, M.D.

Diet and Prevention of Coronary Heart Disease and Cancer, edited by B. Halpern *et al.*, Raven Press, New York, 1986, pp. 95–111.

Other support: National Heart, Lung and Blood Institute.

From the Division of Endocrinology and Metabolism, Department of Medicine, University of California at San Diego, La Jolla.

BINDING OF COAGULATION FACTOR XI TO WASHED HUMAN PLATELETS

The binding of human coagulation factor XI to washed human platelets was studied in the presence of zinc ions, calcium ions, and high molecular weight kininogen. Saturable binding of factor XI occurred at physiological levels of these metal ions when high molecular weight kininogen was present. Binding required platelet stimulation and was specific, reversible, and saturable. Scatchard analysis of the binding data revealed approximately 1500 binding sites per platelet with an apparent dissociation constant of approximately 10 nM. Since this concentration of factor XI is [illegible] platelets [illegible] nM, it suggests that unstimulated factor XI [illegible] stimulated platelets

Other support:

From the

IV. *Neuropharmacology and Physiology*

NICOTINE ALTERS CATECHOLAMINES AND ELECTROCORTICAL ACTIVITY IN PERFUSED MOUSE BRAIN

Nicotine differentially altered electrocortical (ECoG) activity and brain catecholamine metabolism in mice (C3H and C57BL) known to differ in behavioral responses to nicotine. Nicotine appeared to produce a concentration dependent desynchronization of ECoG activity in isolated perfused mouse brain (IPMB) from C3H mice. However, a similar increase in HVA production was unchanged in C3H perfused brains while an apparent reduction in 3-methoxy-4-hydroxyphenethyleneglycol (MPHG) was observed. Brain content of norepinephrine and dopamine remained relatively constant in the various regions tested. In IPMB from C57BL mice, nicotine elicited an enhancement of ECoG amplitude which was accompanied by decreased HVA production rates. A similar trend in MHPG production was also observed. These effects were associated with increased levels of norepinephrine and dopamine in various brain regions.

From: W. G. Cornell, K. and Towell, J. F.

Pharmacology, Biochemistry & Behavior **24**:1 99-105, 1986

Other support: National Institute of Drug Abuse

From the School of Pharmacy, University of Colorado, Boulder

ACUTE CONTINUOUS EXPOSURE TO CIGARETTE SMOKE PRODUCES DISCRETE CHANGES IN CHOLECYSTOKININ AND SUBSTANCE P LEVELS IN THE HYPOTHALAMUS AND PREOPTIC AREA OF THE MALE RAT

By means of a Walton Horizontal Smoking Machine, male rats were exposed to the smoke from 6 cigarettes burned in a continuous fashion. Cholecystokinin (CCK) and substance P levels, determined by means of radioimmunoassay, were measured in [illegible] and preoptic regions. Acute continuous exposure to cigarette smoke [illegible] CCK levels in the paraventricular hypothalamic region as well as [illegible]. Pharmacological antagonism [illegible] this treatment resulted in decreased CCK and substance P levels in the medial preoptic region. The results may be interpreted to indicate that CCK and substance P containing neuronal systems [illegible] cholinergic nicotine-like receptors.

From: K. [illegible]

Acta Physiologica Scandinavica **125**:437-443, 1985

Other support: Svenska Tobaks AB, Stockholm, Sweden

From the [illegible] Histology, Karolinska Institutet, Stockholm, Sweden, Research [illegible] Laboratory, Department of Obstetrics and Gynecology, [illegible] Pharmacy, Faculty of [illegible] and [illegible] Medicine, [illegible]

EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON HYPOTHALAMIC DOPAMINE AND NOREPINEPHRINE CONCENTRATIONS IN THE NEONATAL RAT BRAIN

[illegible]

CLONIDINE TREATMENT ELEVATES CONTENT OF NEUROPEPTIDE Y IN CARDIAC NERVES

[illegible] clonidine lowers blood pressure by means of central inhibition of sympathetic nerve activity. Recently, neuropeptide Y (NPY) has been shown to coexist with noradrenaline (NA) in, for example, cardiovascular nerves and postganglionic sympathetic nerve cell bodies. Drugs, receptors for, have been shown to cause depletion of not only NA but also of NPY. The main peculiarity of NPY-LI is its tissue [illegible] due to increased sympathetic nerve activity. Here we report that these two antihypertensive drugs, both known to impair sympathetic noradrenergic transmission, but via different modes of action, selectively affect the NPY content of [illegible] in opposite directions. Thus, in contrast to the reduction seen after reserpine treatment, the clonidine regimen actually increased the cardiac levels of NPY-LI, which is located in sympathetic nerves.

The consequences of both reserpine and clonidine treatment ultimately would be an impairment of NPY function in the cardiac sympathetic nerves, either due to depletion or reduced release. Since NPY exerts potent vasoconstrictor effects, the present findings and considerations may have bearing on the antihypertensive actions of the drugs as well as rebound phenomena after chronic withdrawal.

[illegible] Acta Physiologica Scandinavica, Vol. [illegible], pp. [illegible], 1986

[illegible] 128, pp. [illegible], 1986.

Other support: The Swedish Medical Research Council, the Swedish Tobacco Company, Petrus and Augusta Hedlunds Stiftelse, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

CAPSAICIN-SENSITIVE NERVES AND URETERIC MOTILITY: OPPOSING EFFECTS OF TACHYKININS AND CALCITONIN GENE-RELATED PEPTIDE

Adanced technique of capsaicin-sensitive sensory nerves is present in the guinea pig ureter. These peripheral branches of sensory neurons contain several peptides including multiple tachykinins (substance P (SP), neurokinin A (NKA), and neuropeptide K (NKK)) and calcitonin gene-related peptide (CGRP). In the present study we have investigated the possible influence of capsaicin-sensitive sensory nerves and [illegible] peptides on ureteric motility *in vivo*.

Capsaicin caused inhibition of the ureteric contractions at 0.1 mmol/kg, which the [illegible] dose (100 nmol/kg) induced an initial inhibition, which was followed by a long-lasting stimulation of motility. The capsaicin effects were absent in capsaicin-pretreated animals. The CGRP inhibited ureteric contractions, while NKA caused a long-lasting stimulation of motility. The CGRP and NKA effects were still present in [illegible] animals. Electrical stimulation of the ureteric nerves in the ganglion [illegible] inhibition of ureteric motility, which was followed by a long-lasting [illegible]. This nerve stimulation evoked response was still present [illegible] capsaicin-pretreated animals, but was [illegible] and abolished [illegible].

[illegible]

Other support: Austrian Scientific Research Funds, the Swedish Medical Research Council, the Swedish Tobacco Company and the Swiss National Science Foundation.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CAPSAICIN INDUCED RELEASE OF MULTIPLE TACHYKININS (SUBSTANCE P, NEUROKININ A AND ELEDOISIN-LIKE MATERIAL) FROM GUINEA PIG SPINAL CORD AND URETER

The release of tachykinins from isolated slice preparations of the guinea-pig spinal cord and ureter was studied *in vitro*. Capsaicin (10 μM) caused release of substance P, neurokinin A and an eledoisin-like component from both the spinal cord and ureter. The release of tachykinins induced by capsaicin or potassium (60 mM) was calcium dependent. No detectable release of neurokinin B or neuropeptide K, an N-terminally extended form of neurokinin A, was induced by capsaicin. No detectable release of tachykinins could be demonstrated after exposure to agents which are known to activate C-fiber afferents, such as histamine, bradykinin, serotonin, prostaglandins E_1, E_2 or acetylcholine. Protein extravasation in the ureter, as determined by the Evans Blue extravasation technique was used as a functional correlate to the tachykinin release. Protein extravasation was induced *in vivo* by local intraluminal injections of capsaicin at several hundred-fold lower concentrations than those required to induce a detectable release of tachykinins *in vitro*. The difference may, however, partly depend on the experimental conditions and the detection limit of the tachykinin assay used. The protein extravasation response to capsaicin was absent after systemic capsaicin pretreatment, which causes a marked depletion of tachykinins in the ureter. In conclusion, capsaicin evokes release of several tachykinins from both central and peripheral endings of primary afferent neurons. The peptides released from sensory nerves in the periphery may induce effects such as protein extravasation and smooth muscle contraction.

Hua, X.-Y., Saria, A., Gamse, R., and *Lundberg, J. M.*

Neuroscience **19**(1):313-319, 1986.

Other support: Swedish Medical Research Council, Austrian Scientific Research Funds, the Swedish Tobacco Company, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

SPECIFIC RECEPTOR AND CARDIOVASCULAR EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE

Specific binding sites for calcitonin gene-related peptide (CGRP) were demonstrated in the rat heart and spleen. Autoradiography revealed rat [^{125}I]iodo CGRP binding associated with the intima and media of the aorta, the coronary arteries and the heart valves, and the red pulp of the spleen. Half-maximal inhibition of rat [^{125}I]iodo-CGRP binding to membranes of the rat atria and the spleen was obtained with, respectively, 5 and 0.35 nM unlabeled rat CGRP; these values correspond to EC_{50} values of 3 and 0.14 nM for activation of adenylate cyclase by CGRP. In the isolated, spontaneously beating right atrium, the EC_{50} values of stimulation of the force and rate of contraction by rat CGRP were 120 and 70 nM, respectively. Rat CGRP caused relaxa-

tion of splenic strips, precontracted with noradrenaline; the EC_x was 50 nM. The β-adrenergic blocking agent metoprolol, while obliterating the increase in the force and rate of contraction evoked by noradrenaline in the right atrium, did not significantly change the action of CGRP. Similarly, preserved action of CGRP in the presence of indomethacin as well as mepyramine and cimetidine argues against a role of prostaglandins or histamine in the functional responses of CGRP. Much like CGRP, capsaicin, which releases mediators from sensory neurons, caused stimulation of the force and rate of contraction of the isolated right rat atrium. After tachyphylaxis to CGRP, the response to noradrenaline was intact, while the positive chronotropic and inotropic effects of capsaicin were suppressed. The results indicate that the cardiac effects of capsaicin may be due to the release of endogeneous CGRP through a local mode of action.

Sigrist, S., Franco-Cereceda, A., Muff, R., and *Lundberg, J. M.*

Endocrinology **119**(1):381-389, 1986.

Other support: Swiss National Science Foundation, the Swedish Medical Research Council, the Swedish Tobacco Company, the Hedlunds Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

DUAL CAPSAICIN EFFECTS ON URETERIC MOTILITY: LOW DOSE INHIBITION MEDIATED BY CALCITONIN GENE-RELATED PEPTIDE AND HIGH DOSE STIMULATION BY TACHYKININS?

The effects of capsaicin, in relation to substance P (SP), neurokinin A (NKA), neuropeptide K (NPK) and calcitonin gene-related peptide (CGRP) which coexist in local sensory nerves, on the motility of the guinea-pig ureter were studies *in vivo* and *in vitro*. Capsaicin in a *low dose* (10 nmol kg^{-1}) given i.v. inhibited spontaneous, peristaltic contractions, as revealed by perfusion-pressure changes of the constantly perfused ureter *in vivo*. This action was independent of autonomic reflexes and prostaglandin formation. Capsaicin stimulated ureteric motility at *higher doses* (100 and 500 nmol kg^{-1}). The dual effects of capsaicin on the ureteric contractility were absent 2 weeks after systemic capsaicin treatment, which depletes sensory neuropeptides. Both NKA and NPK initiated, as well as increased, the magnitude of the peristaltic contractions of the ureter, while SP only caused a minor excitatory effect. The CGRP inhibited spontaneous, as well as NKA- and NPK-induced ureteric peristaltic contractions. *In vitro* experiments on the ureter revealed that capsaicin (10^{-6} M) induced phasic circular muscle contractions in 60% of the experiments. Neurokinin A, NPK and SP consistently increased the contractile activity. The NKA tachyphylaxis inhibited the contractile response to other tachykinins and capsaicin. The SP analogue Spantide (/D-Arg^1, D-$Trp^{7,9}$, Leu^{11}/-SP) inhibited the contractile responses to SP, NKA and NPK. The CGRP also inhibited the NKA- and NPK-induced contractions of the ureter *in vitro*. In conclusion, capsaicin, which induces the release of mediators from sensory nerves within the ureter, has either stimulatory or inhibitory effects on ureteric smooth muscle, depending on the *in vivo* dose administered. The inhibitory response at a low capsaicin dose is similar to the effect of CGRP, while the contractile effects at higher doses resemble the response to tachykinins.

Hua, X.-Y. and *Lundberg, J. M.*

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Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Swedish Work Environment Fund, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CO-RELEASE OF NEUROPEPTIDE Y AND CATECHOLAMINES UPON ADRENAL ACTIVATION IN THE CAT

The release of neruopeptide Y (NPY)-like immunoreactivity (-LI) in relation to catecholamines from the cat adrenal was studied in anaesthetized animals. Abdominal surgery increased plasma levels of NPY-LI from 65 ± 6 to 149 ± 26 pmol l^{-1}. A positive veno–arterial concentration gradient over the adrenal gland was found for both NPY-LI, adrenaline (Adr) and noradrenaline (NA) during basal conditions. Asphyxia for 2 min increased the output of both NPY-LI and catecholamines from the adrenal. Electrical stimulation of the splanchnic nerve caused a marked increase in adrenal output of NPY-LI and catecholamines. The adrenal content of NPY-LI, as well as the release of NPY-LI from the adrenal, was at least 1000-fold lower on a molar basis than that of catecholamines. The concentration of NPY-LI in the adrenal vein upon splanchnic nerve stimulation was in the nM range. Reversed-phase HPLC characterization revealed that NPY-LI in the adrenal, and in the adrenal venous plasma collected during splanchnic nerve stimulation, was closely related to synthetic porcine NPY. Stimulation with bursts of 20 Hz for 1 s with 10 s intervals for 2 min caused a four-fold higher output of NPY-LI and Adr compared to a continuous stimulation with 2 Hz, giving the same number of impulses. The NA output, however, was only slightly increased by burst stimulation. Guanethidine did not reduce the adrenal output of NPY-LI or catecholamines induced by splanchnic nerve stimulation, while the release was abolished by chlorisondamine. NPY enhanced the *in vitro* contractile activity of Adr and NA on the a. profunda femoris from the cats used in the functional experiments. The threshold concentration of NPY for this enhancement was 250 pM. The NPY in higher concentrations (> 10^{-8} M) had contractile effects *per se*. In conclusion, the present data suggest a functional relationship between adrenal release and effects of NPY and Adr.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica **126**:231-238, 1986.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CO-RELEASE OF NEUROPEPTIDE Y AND CATECHOLAMINES DURING PHYSICAL EXERCISE IN MAN

Venous plasma levels of neuropeptide Y-like immunoreactivity (with chromatographic properties of synthetic neuropeptide Y) increased in parallel with catecholamines, heart rate and blood pressure during graded physical exercise in man. The plasma levels of neuropeptide Y correlated better with the levels of noradrenaline than

adrenaline, suggesting release of a neural origin. Taken together with previous results, this suggests that neuropeptide Y is released together with noradrenaline upon sympathetic activation during physiological conditions in man. Determinations of plasma neuropeptide Y may therefore be valuable in the assessment of sympathetic nerve activity.

Lundberg, J. M., et al.

Biochemical and Biophysical Research Communications **133**(1):30-36, November 27, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Karolinska Institute, the Swedish National Association Against Heart and Chest Diseases, and the Research Council of the Swedish Sports Federation.

From the Departments of Pharmacology, Karolinska Institute, Stockholm, Sweden.

MECHANISMS UNDERLYING PRE- AND POSTJUNCTIONAL EFFECTS OF NEUROPEPTIDE Y IN SYMPATHETIC VASCULAR CONTROL

The effects of porcine neuropeptide Y (NPY) regarding sympathetic vascular control were studied *in vitro* on isolated rat blood vessels. The 10^{-8} M NPY enhanced (about two-fold) the contractile responses to transmural nerve stimulation (TNS), noradrenaline (NA) and adrenaline (about two-fold) in the femoral artery. Higher concentrations of NPY (> 10^{-8} M) caused an adrenoceptor-resistant contraction *per se*. The TNS-evoked [^{3}H]NA efflux was significantly reduced by NPY in a concentration-dependent manner (threshold 10^{-9} M). The calcium antagonist, nifedipine, abolished the contractile effects of NPY and the NPY-induced enhancement of NA contractions but did not influence the prejunctional inhibition of [^{3}H]NA release. Receptor-binding studies showed that the ratio of alpha$_1$- to alpha$_2$-adrenoceptors in the femoral artery was 30:1. The NPY did not cause any detectable change in the number of alpha$_1$- or alpha$_2$-adrenoceptor binding sites or in the affinity of alpha$_2$-binding sites, as revealed by prazosin- and clonidine-binding, respectively. The NPY also inhibited the TNS-evoked [^{3}H]NA release (by 42-86%) in the superior mesenteric and basilar arteries and in femoral and portal veins. The NPY still depresed TNS-evoked [^{3}H]NA secretion from the portal vein in the presence of phentolamine. The NPY caused a clear-cut contraction in the basilar artery, increased the contractile force of spontaneous contractions in the portal vein, while only weak responses were observed in the superior mesenteric artery and femoral vein. The NA-induced contraction was markedly enhanced by NPY in the superior mesenteric artery, only slightly enhanced in the portal vein and uninfluenced in the femoral vein. In conclusion, in all blood vessels tested, NPY depresses the TNS-evoked [^{3}H]NA secretion via a nifedipine-resistant action. Furthermore, NPY exerts a variable, CA^{2+}-dependent vasoconstrictor effect and enhancement of NA and TNS contractions.

Pernow, J., Saria, A. and *Lundberg, J. M.*

Acta Physiologica Scandinavica **126**:239-249, 1986.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Karolinska Institute and the Austrian Scientific Research Fund.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

EFFECTS OF VIP, PHM AND SUBSTANCE P ON BLOOD VESSELS AND SECRETORY ELEMENTS OF THE HUMAN SUBMANDIBULAR GLAND

The effects of the neuropeptides VIP, PHM and substance P (SP) on vascular smooth muscle tone, K^+ secretion from exocrine elements and tissue content of cyclic AMP (cAMP) in the human submandibular gland were studied *in vitro*.

All three peptides caused relaxation of nonradrenaline contracted human submandibular arteries at nM concentrations. SP was slightly more active than VIP and PHM which had a similar potency as vasodilators. Only carbachol but not VIP, PHM or Sp stimulated K^+ secretion from exocrine elements of the human submandibular gland. Principally similar *in vitro* effects on K^+ secretion were obtained on the cat submandibular gland but in the rat not only carbachol but also SP stimulated K^+ secretion. VIP and PHM increased cAMP production of exocrine elements in the human submandibular gland in nM concentrations. VIP was about 5-fold more potent than PHM with regards to cAMP production.

In conclusion, VIP, PHM and SP relaxed human submandibular arteries in vitro. Both VIP and PHM stimulated cAMP production in glandular tissue but none of the three peptides induced K^+ secretion from human submandibular gland tissue. This suggests that, in contrast to the situation in the rat, SP does not cause watery salivation in man, while VIP and PHM may modulate protein e.g., amylase content of the saliva.

Larsson, O., Duner-Engstrom, M., *Lundberg, J. M.*, and Fredholm, B. B.

Regulatory Peptides **13**:319-326, 1986.

Other support: Swedish Medical Research Council and the Swedish Tobacco Company.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

NEUROPEPTIDE Y-A COTRANSMITTER WITH NORADRENALINE AND ADENOSINE 5'-TRIPHOSPHATE IN THE SYMPATHETIC NERVES OF THE MOUSE VAS DEFERENS? A BIOCHEMICAL, PHYSIOLOGICAL AND ELECTROPHARMACOLOGICAL STUDY

A combination of biochemical, physiological and electropharmacological methods was employed to examine the occurrence of neuropeptide Y and the pre- and postjunctional effects of this peptide on sympathetic neuromuscular transmission in the mouse vas deferens. This tissue had a high content of neuropeptide Y-[illegible] immunoreactive material, suggesting a dense innervation by neuropeptide Y-containing nerve fibres. Addition of neuropeptide Y at concentrations from 5×10^{-10} to 5×10^{-7} M induced both pre- and postjunctional effects *in vitro*. Neuropeptide Y *per se* induced a rise in the resting tension, and "instantly" potentiated the contractile effects of exogenous noradrenaline and of the stable adenosine 5'-triphosphate (ATP) analogue, α,β-methylene ATP. Neuropeptide Y reduced the secretion of [^{3}H]noradrenaline evoked by electrical nerve stimulation, and selectively depressed the stimulus-evoked, but not the spontaneously occurring excitatory junction potentials in smooth muscle cells. Further, neuropeptide Y reduced the amplitudes of the twitch contractions evoked by electrical field stimulation with short stimulus trains at 10 Hz, and also (although to a smaller extent) the delayed contractile response to longer trains of nerve stimuli. The

pre- and postjunctional effects of neuropeptide Y were not changed by α- or β-adrenoceptor blocking agents, or by tachyphylaxis to the effects of ATP, or by the calcium-channel blocker nifedipine.

In conclusion, sympathetic neuromuscular transmission in the mouse vas deferens may be mediated not only by noradrenaline and ATP, but also by neuropeptide Y. This peptide may play a dual role: initially to locally potentiate the contractile response to noradrenaline and ATP, and subsequently to locally "turn off" the secretion of transmitter in previously (hyper-?)active regions of the nerve terminals.

Stjärne, L., *Lundberg, J. M.*, Åstrand, P.

Neuroscience **18**(1):151-166, 1986.

Other support: Swedish Medical Research Council and the Swedish Tobacco Company.

From the Departments of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden.

IS SYNAPTIC TRANSMISSION MODULATED BY PROGESTERONE?

The influence of the gonadosteroid hormone progesterone on synaptic transmission was studied using the frog neuromuscular preparation. Intracellular recording of synaptic potentials revealed enhanced release of acetylcholine from motor nerve terminals exposed to progesterone (3nM-3mM). The following effects were observed. (1) An augmented quantal content of evoked release of transmitter; (2) an elevation in synaptic facilitation; and (3) a substantial increase in the rate of spontaneously occurring miniature endplate potentials. It is suggested that synaptic transmission at the neuromuscular junction may be naturally modulated by the physiologically oscillating level of progesterone.

Meiri, H. (*Rahamimoff, R.*)

Brain Research **385**:193-196, 1986.

Other support: Muscular Dystrophy Association and the U.S.-Israel Binational Science Foundation.

From the Laboratory of Cell Biology, Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

EFFECT OF *L*-METHIONINE ON CONTRACTILE RESPONSE, CALCIUM INFLUX AND CALCIUM CHANNEL BLOCKING AGENTS IN THE RAT AORTA

L-Methionine incubated with aorta strips and S-adenosyl-*L*-methionine incubated with aorta membranes methylate membrane phospholipids. *L*-Methionine enhances the contractile response of helical strips of rat aorta to KCl. *L*-Methionine also enhances the slow component of the contractile response of rat aorta to norepinephrine associated with influx of exogenous calcium. *L*-Homocysteinethiolactone inhibits

methylation of membrane phospholipids and depresses the contractile response to KCl and to norepinephrine. *L*-Methionine enhances and *L*-homocysteinethiolactone depresses KCl-stimulated influx of calcium into rat aorta strips. *L*-Methionine has no effect on calcium efflux. Tested against calcium channel blocking agents, *L*-methionine reduces the inhibition caused by diltiazem and chlorpromazine but not that caused by TMB 8 or verapamil. It is postulated that methylated intermediates of phospholipd methylation enhance the function of membrane calcium channels.

Landon, E. J., Owens, L., and *Sastry, B. V. R.*

Pharmacology **32**:190-201, 1986.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REGULATION OF ACETYLCHOLINE RELEASE FROM RODENT CEREBRUM BY PRESYNAPTIC RECEPTORS, METHIONINE ENKEPHALIN AND SUBSTANCE P

The findings of this study indicate the operation of a homeostatic relationship between the release of ACh, MEK and SP. This relationship may be of physiological importance in the regulation of the release of ACh. If the amount of ACh in the synaptic gap is low, a positive feedback loop is triggered, causing the release of SP either directly or by a disinhibition phenomenon. Low ACh does not stimulate the negative feedback loop. Hence, no MEK is released, leading to a relief of an inhibitory action of MEK on the release of SP. The released SP would increase further release of ACh by increasing the uptake of Ca^{++}. This process would continue until a peak release of ACh was reached. At this point, the high amount of ACh in the synaptic gap would trigger a negative feedback loop, inducing the release of MEK, which in turn would limit any further release of ACh and SP by decreasing the uptake of Ca^{++}. These feedback systems seem to operate through two different types of muscarinic receptors.

Sastry, B. V. R.

Advances in Behavioral Biology **30**:1047-1056, 1986.

Other support: National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REVIEW: CHOLINERGIC SYSTEMS AND MULTIPLE CHOLINERGIC RECEPTORS IN OCULAR TISSUES

Acetylcholine (ACh), choline acetyltransferases and cholinesterases occur in cornea, iris-ciliary body complex and retina of several vertebrates. In cornea, ACh may serve as a sensory transmitter as well as a local hormone, the function of which is

not delineated. The function of ACh as the parasympathetic neurotransmitter at the iris and ciliary body is well established. The muscarinic receptors on the iris smooth muscle are similar to the muscarinic receptors (M2 type in two way classification) at other smooth muscles towards their interaction with agonists and antagonists. Binding studies using radiolabeled antagonists and their displacement by agonists indicate that muscarinic receptors in membranes of iris-ciliary body complex are heterogeneous indicating more than one subtype of muscarinic receptors. A subtype other than M2 receptors may occur at the presynaptic sites of parasympathetic nerves, which have yet to be investigated using specific agonists and antagonists. Cholinergic markers, choline acetyltransferase and acetylcholinesterase, differ quantitatively and qualitatively in retinas of different species. However, amacrine cells are cholinergic in all vertebrate species. Although they make up 1% of retinal neurons, they influence the activity of a majority of ganglion cells. Cholinergic effects in ganglia are mediated through nicotinic and muscarinic receptors. Both of these types of cholinergic receptors are heterogeneous. They have yet to be investigated for their subtypes using specific agonists and antagonists. Although the role of cholinergic retinal neurons in the processing of visual information is not known, their input to ganglion cells generally increases the rate of spontaneous activity or the number of action potentials in light-evoked responses. Thus, the cholinergic input seems to modify the overall neuronal input to the ganglion cells from the receptive fields. Endothelial cells of blood vessels contain muscarinic receptors, which are activated by ACh to cause relaxation. Although retinal blood vessels provide recognizable characteristic signs in diabetes mellitus and hypertensive disease, no information is available on the muscarinic receptors of these vessels.

Sastry, B. V. Rama

The Journal of Ocular Pharmacology **1**(2):201-226, 1985.

Other support: U. S. Public Health Service and the National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

KINETICS OF [^{3}H]MPP$^+$ UPTAKE IN DOPAMINERGIC NEURONS OF MOUSE: REGIONAL EFFECTS OF MPTP NEUROTOXICITY

In an effort to determine the specificity of MPTP/MPPP$^+$ toxicity with respect to the dopaminergic systems, the effect of prior MPTP treatment on [^{3}H]MPP$^+$ uptake in the striatum and olfactory tubercle of BALB/cBy mice was examined. Kinetic analysis of [^{3}H]MPP$^+$ uptake indicated a reduction of V_{max} values in both striatum (49%, $P<0.05$) and olfactory tubercle (26%, $P<0.05$). MPTP treatment did not significantly alter the K_m in either region, although MPP$^+$ accumulates in both olfactory tubercle and striatum, these dopaminergic systems show different sensitivity to the neurotoxicity of MPTP/MPP$^+$.

Sershen, H. et al.

European Journal of Pharmacology **126**:337-339, 1986.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, NY.

EFFECT OF AMPHETAMINE ON 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) NEUROTOXICITY IN MICE

Amphetamine has been shown to either potentiate or protect against MPTP neurotoxicity. The time course of changes in dopamine and its metabolites was examined after MPTP, amphetamine, or MPTP plus amphetamine administration. Results suggest that under conditions of granular depletion and release of dopamine by 10 mg/kg amphetamine, increased MPTP neurotoxicity occurs. Amphetamine injections at 2-5 mg/kg prevents the decline in dopamine possibly by blockade of the uptake of MPP^+, rather than by an inhibition of monoamine oxidase.

Sershen, H. et al.

Neuropharmacology **25**(8):927-930, 1986.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, NY.

EFFECT OF NICOTINE AND AMPHETAMINE ON THE NEUROTOXICITY OF *N*-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) IN MICE

The present results show the potentiating effect of amphetamine on the ability of MPTP to destroy dopaminergic neurons in striatum of the mouse. A single injection of MPTP (8 mg/kg, retro-orbital) reduced the binding of [^{3}H]mazindol, a marker for dopamine terminals, by 24%. When D-amphetamine (10 mg/kg, s.c.) was given 20 min prior to MPTP, the binding of [^{3}H]mazindol, measured 3-5 days later, was reduced by 58%. It is proposed that the mechanism of this potentiation primarily involves an increased release of dopamine by D-amphetamine, and free radical-mediated processes. Although nicotine also releases dopamine from the striatum, no effect was observed when it was administered prior to MPTP. The lack of effect is probably related to short duration of action of nicotine and the modest effect on release of dopamine as compared to that of amphetamine.

Sershen, H. et al.

Neuropharmacology **25**(11):1231-1234, 1986.

From the Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

SUPEROXIDE RADICAL-MEDIATED ALTERATION OF SYNAPTOSOME MEMBRANE STRUCTURE AND HIGH-AFFINITY γ-[^{14}C]AMINOBUTYRIC ACID UPTAKE

Mouse cortical synaptosomal structure and function are altered when exposed to hypoxanthine/xanthine oxidase (HPX/XOD)-generated active oxygen/free radical species. The structure of both the synaptic vesicle and plasma membrane systems are altered by HPX/XOD treatment. The alteration of synaptic vesicle structure is exhib-

ited by a significant increase in the cumulative length of nonsynaptic vesicle membrane per nerve terminal. With respect to the nerve terminal plasma membrane, the length of the perimeter of the synaptosome is increased as the membrane pulls away from portions of the terminal in blebs. The functional lesion generated by HPX/XOD treatment results in a reduction in selective high-affinity γ-[^{14}C]aminobutyric acid (GABA) uptake. Kinetic analysis of the reduction in high-affinity uptake reveals that the V_{max} is significantly altered whereas the K_m is not. Preincubation with specific active oxygen/free radical scavengers indicates that the superoxide radical is directly involved. This radical, most probably in the protonated perhydroxyl form, initiates lipid peroxidative damage of the synaptosomal membrane systems. Low-affinity [^{14}C]GABA transport is unaltered by the HPX/XOD treatment. The apparent ineffectiveness of free radical exposure on low-affinity [^{14}C]GABA transport coupled with its effectiveness in reducing high-affinity transport supports the idea that two separate and different amino acid uptake systems exist in CNS tissue, with the high-affinity being more sensitive (lipid-dependent) and/or more energy-dependent (Na^+,K^+-ATPase) than the low-affinity system.

Debler, E. A., *Sershen, H.*, Lajtha, A. and Gennaro, J. F., Jr.

The Journal of Neurochemistry **47**(6):1804-1813, 1986.

From the Center for Neurochemistry, The Nathan S. Kline Institute for Psychiatric Research, Ward's Island; and Department of Biology, New York University, New York.

V. *Pharmacology and Biochemistry*

INHIBITION OF SERINE PROTEASES BY PEPTIDYL FLUOREMETHYL KETONES

We have synthesized peptidyl fluoromethyl ketones that are specific inhibitors of the serine proteases α-chymotrypsin and porcine pancreatic elastase. By analogy with the corresponding aldehydes it is assumed that the fluoromethyl ketones react with the γ-OH group of the active site serine to form a stable hemiacetal. ^{19}F NMR studies of the chymotrypsin-bound trifluoromethyl ketone inhibitors Ac-Leu-*ambo*-Phe-CF_3 and Ac-*ambo*-Phe-CF_3 clearly indicate that the carbonyl carbon is tetrahedral at the active site of the enzyme. The inhibitor is bound as either the stable hydrate or the hemiacetal, involving the active site serine. The effect of varying the number of amino acid residues in the peptidyl portion of the inhibitor and the number of fluorines in the fluoromethyl ketone moiety is examined. In the series of trifluoromethyl ketone elastase inhibitors, the lowering of K_i concomitant with the change from a dipeptide analogue to a tetrapeptide analogue (Ac-Pro-*ambo*-Ala-CF_3, $K_i = 3 \times 10^{-3}$ M; Ac-Ala-Ala-Pro-*ambo*-Ala-CF_3, $K_i = 0.34 \times 10^{-4}$ M) correlates well with the variation in V/K for hydrolysis of the corresponding amide substrates. This trend is indicative of the

inhibitors acting as transition-state analogues. In addition to chain length, the number of fluorine substituents also effects the K_i. In the case of chymotrypsin, the K_i for Ac-Leu-*ambo*-Phe-CF_3 is 30-fold lower than that for Ac-Leu-*ambo*-Ala-CF_2H (0.88×10^{-4} M vs 25×10^{-4} M). With elastase this trend is not as profound. In all cases, however, the difluoro- and trifluoromethyl ketones are better inhibitors than the monofluoromethyl and nonfluorinated analogues. This improvement must be associated with both the degree of hydration of the fluoromethyl ketones and the significant effect that fluorine substitution has on lowering the first pK_a of the hemiacetal hydroxyl. The latter change would cause the more fluorinated inhibitor to be able to interact better with the anionic hole near the active site. Fluorine substitution also lowers the k_{off} values for the inhibitors. With elastase the trifluoromethyl ketone tetrapeptide has a k_{off} of 1.25×10^{-5} s^{-1}, while the corresponding difluoromethyl compound has a k_{off} of 0.007 s^{-1}. The monofluoromethyl ketone inhibitor of chymotrypsin, Ac-Leu-*ambo*-Phe-CFH_2, is a weak competitive inhibitor ($K_i = 200 \times 10^{-4}$ M). It also demonstrates time-dependent irreversible inhibition with a second-order rate constant of 1.7 M^{-1} s^{-1}. The irreversible inhibition is accompanied by covalent modification of a histidine residue and by fluoride ion release as detected by ^{19}F NMR spectroscopy.

Imperiali, B. and *Abeles, R. H.*

Biochemistry **25**(13):3760-3767, 1986.

Other support: National Institutes of Health.

From the Graduate Department of Biochemistry, Brandeis University, Waltham, MA.

A VERSATILE SYNTHESIS OF PEPTIDYL FLUOROMETHYL KETONES

Peptidyl fluoromethyl ketones constitute an important class of inhibitors of a number of serine proteases such as chymotrypsin and elastase. This communication presents a general method for the synthesis of these peptide analogs. The standard protocol described can be used for the synthesis of many analog compounds which can be tested against specific serine proteases by the selection of an appropriate nitroalkane and suitably protected peptide component.

Imperiali, B. and *Abeles, R.*

Tetrahedron Letters **27**(2):135-138, 1986.

Other support: National Institutes of Health.

From the Department of Biochemistry, Brandeis University, Waltham, MA.

BARREL ROTATION AND PROSTRATION BY VASOPRESSIN AND NICOTINE IN THE VESTIBULAR CEREBELLUM

The aim of this study was to determine whether the primary sites for the action of vasopressin and nicotine in producing barrel rotation and prostration in rats were located in the modular cerebellum, *i.e.*, lobule X. When arginine vasopressin was

administered into either the fourth ventricles or directly into the nodular cerebellum via chronically implanted cannulae, the rats displayed intermittent barrel rotation and clonic convulsions. The administration of nicotine into the same areas resulted in prostration, atonia and, occasionally, clonic convulsions. A few days after the nodular cerebellum was lesioned with kainic acid, the motor distrubances resulting from either agent were virtually abolished. Histologic studies revealed that kainic acid had destroyed Purkinje and other large neurons, but had left the granular neurons relatively intact. The administration of procaine into either the fourth ventricles or nodular cerebellum blocked the behavioral responses of either vasopressin or nicotine given into the fourth ventricles. It was concluded that the nodular cerebellum is a primary site for the motor disturbances produced by vasopressin and nicotine.

Maiti, A., Salles, K. S., Grassi, S., and *Abood, L. G.*

Pharmacology, Biochemistry & Behavior **25**:583-588, 1986.

Other support: Department of Health and Human Services.

From the Center for Brain Research, University of Rochester Medical Center, Rochester, NY.

BEHAVIOR AND RECEPTOR CHANGES AFTER KAINATE LESIONING OF NODULAR CEREBELLUM

A study was undertaken on the effects of kainic acid lesioning on the nodulus of the rat cerebellum on behavior and various brain receptors in conscious, freely moving rats. The basis for the study was the observation that barrel rotation and other motor effects induced by intraventricular administration of vasopressin and nicotine could be elicited by their administration into the nodular area of the cerebellum. Histology revealed a marked destruction of Purkinje, stellate, and Golgi cells in the area surrounding the site of kainate administration, with little effect on the granular cells. Immediately after administering 4–12 ng of kainic acid into the nodular cerebellum, rats exhibited circling movements, barrel rotation, and clonic convulsions accompanied by stereotypic head movements, aggressiveness, and gnawing-biting; effects gradually diminishing over 3 days. Receptor binding studies 4–14 days after kainate lesioning revealed a marked increase in ^{3}H-nicotine and ^{3}H-QNB binding in the surrounding cerebellar region, caudate nucleus, and hypothalamus, with no change in ^{3}H-dihydromorphine binding. The findings are consistent with the hypothesis that nicotinic and muscarinic pathways in the vestibular cerebellum, along with its connection to nigrostriatal dopaminergic systems, are involved in the mediation of barrel rotation, ataxia, and other motor disturbances resulting from administration of vasopressin or nicotine intraventricularly.

Maiti, A., Salles, K. S., Grassi, S., and *Abood, L. G.*

Pharmacology, Biochemistry & Behavior **25**:589-594, 1986.

Other support: Department of Health and Human Services.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

BINDING OF HIGH DENSITY LIPOPROTEIN TO CULTURED FIBROBLASTS AFTER CHEMICAL ALTERATION OF APOPROTEIN AMINO ACID RESIDUES

Cultured extrahepatic cells possess a specific high affinity binding site (receptor) for high density lipoprotein (HDL) that is induced by cholesterol delivery to cells. To characterize the binding recognition site(s) on HDL, the ability of HDL to interact with cultured human fibroblasts was assayed after chemical alteration of specific apoprotein amino acid residues. Reduction and alkylation, acetylation, and cyclohexanedione treatment of HDL$_3$ had little or no effect on its cellular binding. Treatment of HDL$_3$ with tetranitromethane (TNM), however, caused a large dose-dependent decrease in binding, with maximum inhibition at 3 mM. Amino acid analysis of the TNM-treated particles showed specific alteration of tyrosine residues, but sodium dodecyl sulfate-gel electrophoresis demonstrated apoprotein cross-linking coincident with decreased binding. These results suggest that modification of HDL tyrosine residues and/or cross-linking of HDL apoproteins alters the ligand site recognized by the HDL receptor. Gradient gel electrophoresis, molecular sieve chromatography, and electron microscopy showed only minor changes in size distribution and shape of HDL$_3$ particles after treatment with 3 mM TNM, but at higher TNM concentrations, coalescence and aggregation of particles was evident. Treatment of HDL$_3$ with 3 mM TNM affected neither its promotion of the low affinity (receptor-independent) cholesterol efflux from cells nor its ability to accept cholesterol from an albumin suspension, yet promotion of high affinity (receptor-dependent) cholesterol efflux from cells was abolished. The finding that TNM treatment of HDL$_3$ decreases both its receptor binding and its promotion of cholesterol efflux from cells without substantial alteration of its physical properties supports the hypothesis that the HDL receptor functions to facilitate cholesterol transport from cells.

Brinton, E. A., Oram, J. F., Chen, C.-H., *Albers, J. J.*, and Bierman, E. L.

The Journal of Biological Chemistry **261**(1):495-503, 1986.

Other support: National Institutes of Health, National Research Service Award Fellowship, and R. J. Reynolds Industries, Inc.

From the Division of Metabolism, Endocrinology and Nutrition, University of Washington, Seattle.

KINETIC STUDIES OF THE REDUCTION OF NEUTROPHIL CYTOCHROME *b*-558 BY DITHIONITE

The reduction with dithionite of neutrophil cytochrome *b*-558, implicated in superoxide generation by activated neutrophils, was investigated by a stopped-flow technique in non-ionic-detergent extracts of the membranes and in crude membrane particles. The dependence of the pseudo-first-order rate constants on the concentration of dithionite was consistent with a mechanism of reduction that involves the dithionite anion monomer $SO_2^{\cdot-}$ as the reactive species. The estimated second-order rate constant was $7.8 \times 10^6 M^{-1} \cdot s^{-1}$ for Lubrol PX-solubilized cytochrome *b*-558 and $5.1 \times 10^6 M^{-1} \cdot s^{-1}$ for the membrane-bound protein. The similarity of the kinetic constants suggests that solubilization did not introduce gross changes in the reactive site. Imidazole and *p*-chloromercuribenzoate, known as inhibitors of NADPH oxidase, did not affect signi-

ficantly cytochrome *b*-558 reduction rates. The reaction rate of cytochrome *b*-558 with dithionite exhibited a near-zero activation energy. The first-order rate constant for reduction decreased with increasing ionic strength, indicating a positive effective charge on the reacting protein.

Aviram, I. and Sharabani, M.

Biochemical Journal **237**:567-572, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, University of Tel Aviv, Tel Aviv, Israel.

ACTIVATION-DEPENDENT REDISTRIBUTION OF CELLULAR COMPONENTS ALTERS SUSCEPTIBILITY OF HUMAN NEUTROPHILS TO CROSS-LINKING AGENTS

Contrary to resting cells, neutrophils stimulated with concanavalin A resist inhibition by bifunctional N-hydroxysuccinimide esters. Con A prestimulated, cross-linker-treated cells released superoxide upon restimulation with PMA but did not respond to chemotactic peptides. Although rates of PMA-elicited NADPH oxidase activity were lowered by the treatment, the activation parameters, namely lag times of the reaction, were not altered. The protection by Con A against blockade by cross-linkers developed concomitantly to the activation of NADPH oxidase and indicated redistribution of cross-linker-susceptible cellular components responsible for activation with PMA. The identity of these components is discussed.

Aviram, I. and Sharabani, M.

Inflammation **10**(3):233-242, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

A POSSIBLE ROLE FOR PROTEIN PHOSPHORYLATION IN THE ACTIVATION OF THE RESPIRATORY BURST IN HUMAN NEUTROPHILS

Two-dimensional gel electrophoresis was used to study protein phosphorylation in granules, membranes, and soluble fractions from human neutrophils that had been loaded with $^{32}P_i$. In resting cells, label was incorporated primarily into proteins of the membranes and the soluble supernatant; little appeared in the granules. Activation of ^{32}P-loaded neutrophils resulted in an increase in the ^{32}P content of a small number of membrane and soluble proteins without a change in the labeling of the granule fraction. the identity of the proteins affected by activation depended on the activating agent used; all of the activating agents, however, caused an increase in the labeling of a group of ~48-kDa proteins that appeared to be distributed between the membranes and the soluble supernatant.

To investigate the role of phosphorylation in the activation of the respiratory burst oxidase, the incorporation of ^{32}P into phosphoproteins was studied in neutrophils from

patients with chronic granulomatous disease. When these cells were exposed to phorbol myristate acetate, one of the agents used for the activation of normal neutrophils, the 48-kDa proteins in the membranes and supernatants failed to take up additional ^{32}P. Phosphorylation patterns in normal neutrophils activated under nitrogen were similar to the patterns seen with cells activated in air, suggesting that the differences in phosphorylation between normal and chronic granulomatous disease neutrophils did not represent secondary effects of the oxidants produced by the normal cells, but reflected primary biochemical differences between the normal and the defective phagocytes. We postulate from these results that the uptake of phosphate by the 48-kDa protein group may be involved in the activation of the respiratory burst oxidase.

Hayakawa, T., Suzuki, K., Suzuki, S., and *Babior, B. M.*

The Journal of Biological Chemistry **261** (20):9109-9115, 1986.

Other support: U. S. Public Health Service.

From the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA.

THE RESPIRATORY BURST OXIDASE OF HUMAN NEUTROPHILS

A superoxide-forming oxidase from activated human neutrophil membranes was solubilized by two slightly different methods, then purified by "dye-affinity" chromatography. Kinetic studies of the purified preparations gave V_{max} values of 5–10 μmol of O_2^-/min/mg of protein, and K_m values for NADH and NADPH that were in reasonable agreement with values determined previously using particulate and crude solubilized preparations of the respiratory burst oxidase. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed prominent bands at 67, 48, and 32 kDa, together with some minor contaminants, whereas gel electrophoresis under non-denaturing conditions gave a single major band that when eluted and re-electrophoresed in the presence of sodium dodecyl sulfate showed bands at 67, 48, 32 kDa. We believe that all three bands represent oxidase components. The flavin content of the purified enzyme was 20.4 ± 2.0 S.E. pmol of FAD/μg of protein, whereas heme averaged 0.1 ± 0.02 pmol/μg and ubiquinone could not be detected. Assuming that the enzyme is composed of one 67-kDa subunit, one 48-kDa subunit, and one 32-kDa subunit (*i.e.*, that its molecular mass is ~150 kDa), it can be calculated to have a turnover number of 700–1500 min^{-1}, in agreement with a value reported previously for oxidase in a particulate O_2^--forming system (Cross, A. R., [illegible], J. J., and Jones, O. T. G. (1985) *Biochem. J.* 226, 881–884), and to contain the following quantities of redox carriers (mol/mol): FAD, 3.0; heme, 0.015; ubiquinone, <0.06. It remains to be determined whether this preparation represents the complete respiratory burst oxidase or is only the pyridine nucleotide dehydrogenating component of a more complex enzyme.

Glass, G. A. DeLisle, D. M., DeTogni, P., and *Babior, B. M.*

The Journal of Biological Chemistry **261** (28):13247-13251, 1986.

Other support: National Institutes of Health.

From the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA.

DIETARY NICOTINE AND ITS SIGNIFICANCE IN STUDIES ON TOBACCO SMOKING

There is increasing interest in ingestion of nicotine by nonsmokers, and popular assumption is that inhalation of tobacco smoke is the sole source of this alkaloid in body fluids of nonsmokers. However, sources other than tobacco (particularly dietary) have been largely overlooked. In the present study, we measured nicotine levels in various solanaceae (tomato, pepper and eggplant) utilizing radioimmunoassay in order to understand the role of these food sources in the ingestion of nicotine. Our findings showed large quantities of nicotine in ripe fruit, and even greater amounts in unripe fruit.

Castro, A. and Monji, N.

Biochemical Archives **2**(2):91-97, 1986.

From the Department of Pathology, University of Miami School of Medicine, Miami, FL, and Genetics Systems Corp., Seattle.

IRON MEDIATES PARAQUAT TOXICITY IN *ESCHERICHIA COLI*

The role of iron ions in paraquat toxicity was studied in bacterial system. We show that addition of ferrous iron led to an enhancement of the bacterial killing, whereas addition of chelating agents, such as nitrilotriacetate and desferrioxamine, markedly reduced, up to a total abolishment, the toxic effects. The calculated rates of bacterial killing are proportional to both paraquat and iron concentrations, and conform to the rate equation $dN/dt = -k[\text{paraquat}][Fe^{2+}]$. The killing constant for iron, k, is 24-fold smaller than the corresponding value for copper.

Mannitol, an $OH^{\cdot}$ scavenger, has a partial protective effect: 15-35% at concentrations range of 1-50 mM, respectively. Histidine, on the other hand, provided a more efficient protection that may be due to a combination of various effects. Induction of endogenous superoxide dismutase and catalase provided partial protection (about 25%).

These findings, together with an earlier study on the role of copper in paraquat toxicity indicate that transition metals play a central catalytic role in the production of the deleterious effects of paraquat, probably by redox cycling and producing $OH^{\cdot}$ via the site-specific Fenton reaction.

[illegible]bi, P., Kohen, R., Katzhendler, J. and *Chevion, M.*

The Journal of Biological Chemistry **261**(27):12472-12476, 1986.

Other support: United States-Israel Binational Foundation.

From the Departments of Cellular Biochemistry and Pharmaceutical Chemistry, Hebrew University of Jerusalem, Israel.

HUMAN PRION PROTEIN cDNA: MOLECULAR CLONING, CHROMOSOMAL MAPPING, AND BIOLOGICAL IMPLICATIONS

A human complementary DNA whose protein product is considered to be the major component of scrapie-associated fibrils in Creutzfeldt-Jakob disease, kuru, and Gerstmann-Straussler syndrome has been identified and characterized. The extensive

homology of this gene sequence to the hamster PrP 27- to 30-kilodalton prion protein complementary DNA clone, and its existence as a single copy in the human genome, leads to the conclusion that this is the human prion gene. This human prion gene has been mapped to human chromosome 20, negating a direct link between the prion protein and Down's syndrome or the amyloid of Alzheimer's disease.

Liao, Y.-C. J., Lebo, R. V., *Clawson, G. A.* and Smuckler, E. A.

Science **233**:364-367, 1986.

Other support: National Institutes of Health.

From the Department of Pathology, University of California, San Francisco.

p60$^{v\text{-}src}$ ACTIVITY DETECTED IN THE CHROMAFFIN GRANULE MEMBRANE

Using monoclonal antibodies specific for p60$^{v\text{-}src}$ we have detected high levels of this kinase in adrenal medullary chromaffin tissue and in highly purified chromaffin granule (secretory vesicle) membranes. An immune complex kinase assay was applied to fractions of adrenal medullary tissue resolved on sucrose density gradients. Thirty-seven per cent of the total tissue p60$^{v\text{-}src}$ activity was found in association with chromaffin granule or granule membrane markers. Localization of a significant fraction of total cellular p60$^{v\text{-}src}$ activity to this secretory vesicle membrane suggests that the kinase may function in the regulation of neurotransmitter release.

Parsons, S. J. and *Creutz, C. E.*

Biochemical and Biophysical Research Communications **134**(2):736-742, 1986.

Other support: National Institute of Health and University of Virginia Diabetes Research and Training Center.

From the Departments of Microbiology and Pharmacology and the Programs in Neuroscience and Cell and Molecular Biology, University of Virginia, Charlottesville.

PHOSPHORYLATION OF A CHROMAFFIN GRANULE-BINDING PROTEIN IN STIMULATED CHROMAFFIN CELLS

A procedure was devised to determine whether in the stimulated chromaffin cell phosphate is incorporated into specific proteins ("chromobindins") that bind to chromaffin granule membranes in a Ca^{2+}-dependent manner. Cells were preincubated with ^{32}P-labeled orthophosphate, then challenged with secretory stimuli. A postmicrosomal supernatant fraction was prepared from the cells and incubated with unlabeled chromaffin granule membranes in the presence of 5 mM Ca^{2+}. Proteins that bound to the membranes were isolated by centrifugation and examined for ^{32}P content by electrophoresis and autoradiography. Stimulation by carbamylcholine, nicotine, 56 mM K^+, or 2 mM Ba^{2+} led to the incorporation of ^{32}P into a 37-kDa protein that had previously been characterized as a substrate for protein kinase C *in vitro* chromobindin 9, or CB9. Incorporation of ^{32}P into this protein was dependent on extracellular Ca^{2+} and followed a time course that paralleled secretion of catecholamines, returning to base-line levels after 30 min, when secretion terminated. ^{32}P was also incorporated into a 58-kDa

protein that may be tyrosine hydroxylase and into an unidentified 28-kDa protein in response to cell stimulation, but neither of these proteins bound to granule membranes in a Ca^{++}-dependent manner. Treatment of cells with phorbol 12,13-dibutyrate, an activator of protein kinase C, led to ^{32}P incorporation into the 37-kDa protein that was only 30% of the level obtained with nicotinic stimulation, suggesting that additional kinases may be involved in phosphorylating this protein in the stimulated cell.

Michener, M. L., Dawson, W. B., and *Creutz, C. E.*

The Journal of Biological Chemistry **261**(14):6548-6555, 1986.

Other support: National Institutes of Health, the National Science Foundation and the University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology and the Programs in Neuroscience and Cell and Molecular Biology, University of Virginia, Charlottesville.

WHEN DO METAL COMPLEXES PROTECT THE BIOLOGICAL SYSTEM FROM SUPEROXIDE TOXICITY AND WHEN DO THEY ENHANCE IT?

Many copper and iron complexes can be reduced by O_2^- as well as by H_2O_2. According to the rates of reduction and the concentration of O_2^- and H_2O_2, the metal complexes may serve either as catalyst of O_2^- dismutation or as catalysts of the reaction between O_2^- and H_2O_2 to form $OH^\cdot$ radical (Haber-Weiss reaction). Various factors which influence whether metal complexes protect the biological systems from superoxide toxicity or enhance it are discussed.

Czapski, G. and Goldstein, S.

Free Radical Research Communications **1**(3):157-161, 1986.

Other support: G. S. F. Neuherberg, West Germany.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

MECHANISM AND REACTION PRODUCTS OF THE OXIDATION OF Cu(I)-PHENANTHROLINE BY H_2O_2

We have suggested a possible reaction mechanism for the oxidation of the cuprous phenanthroline complex by H_2O_2 in the presence of formate and methanol. The cuprous phenanthroline complex was generated by pulse and γ radiolysis. We measured the decay kinetics of this complex as well as the chain length of this reaction. Our observations indicate that in this reaction $OH^\cdot$ is not formed directly, but through the decomposition of a metal-peroxo complex. This mechanism does not necessarily operate with other copper compounds, especially with copper complexes bound to a biological target.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **1**(5-6):373-380, 1985.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

THE ROLE AND MECHANISM OF METAL IONS AND THEIR COMPLEXES IN ENHANCING DAMAGE IN BIOLOGICAL SYSTEMS OR IN PROTECTING THESE SYSTEMS FROM THE TOXICITY OF O_2^-

Copper complexes of 1,10-phenanthroline and some substituted 1,10-phenanthroline cleave DNA in the presence of a reducing agent and molecular oxygen. Generally, the damage is attributed to hydroxyl radicals which are formed through the Haber-Weiss reaction. It is assumed that this reaction occurs with the ternary metal complexes with the biological target and the mechanism is defined as the "site specific mechanism." In these systems, O_2^- drives the cycle through the reduction of copper(II). On the other hand, these same copper complexes catalyze the dismutation of O_2^- and thus should protect the systems from O_2^- toxicity. In this article, the toxicity of these complexes is explained on kinetic grounds. A general discussion on the various factors which could cause the metal ions or their complexes to act either as protectors from O_2^- toxicity or as sensitizers of toxic effects of O_2^- is given.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **2**:3-11, 1986.

Other support: Israel Academy of Science.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

MECHANISM AND REACTION PRODUCTS OF THE OXIDATION OF CU(I)-PHENANTHROLINE BY H_2O_2

We have suggested a possible reaction mechanism for the oxidation of the cuprous phenanthroline complex by H_2O_2 in the presence of formate and methanol. The cuprous phenanthroline complex was generated by pulse and γ radiolysis. We measured the decay kinetics of this complex as well as the chain length of this reaction. Our observations indicate that in this reaction $OH\cdot$ is not formed directly, but through the decomposition of a metal-peroxo complex. This mechanism does not necessarily operate with other copper compounds, especially with copper complexes bound to a biological target.

Goldstein, S. and *Czapski, G.*

Journal of Free Radicals in Biology & Medicine **1**:373-380, 1985.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

BACTERICIDAL EFFECT OF H_2O_2 AND DNA DAMAGE IN xthA MUTANTS OF *E. COLI*

Hydrogen peroxide is a normal metabolite in aerobic cells. Although not a very toxic species on its own, it may react with transition metals or metal chelates to produce

radicals that have been shown to damage membrane lipids and nucleic acids. *E. coli* cells bearing functional DNA repair genes are resistant to millimolar concentrations of H_2O_2. Endonuclease III deficient (xthA) and recA mutants have been shown to be hypersensitive to H_2O_2. We have used these mutants to investigate the role of endogenous iron and copper in promoting the DNA damaging and bactericidal effects of H_2O_2 on growing bacterial cells.

The key role of transition metal ions in the toxicity of H_2O_2 is well documented. However, addition of various chelating agents, such as EDTA, DTPA and Desferrioxamine to growing xthA cells just before the introduction of H_2O_2 had only a slight protective effect. In contrast, preincubation of these cells with chelators, including OP, followed by washing and resuspension in growth medium had a marked protective effect. Results using cells grown in metal-poor medium showed that killing by H_2O_2 was proportional to the intrinsic iron content of the cells, whereas the killing of resting cells by copper-ascorbic acid was proportional to the amount of Cu^{++} absorbed to the cells.

Treatment of growing cultures of wild type or mutant cells with non-lethal concentrations of H_2O_2 caused an appreciable number of single strand breaks (SSB). With bactericidal concentrations we found a correlation between the killing effect of H_2O_2 or H_2O_2OP and the extent of DNA degradation (SSB and DSB). The hypersensitivity of xthA and recA cells to the bactericidal effect of H_2O_2 correlated with an increase in the number of SSB. Since recA cells are also hypersensitive to H_2O_2 and a great part of the SSB is repairable upon incubation in growth medium, it follows that the repair of the majority of the lesions does not involve exonuclease III which explains the almost normal sensitivity of xthA cells to γ-radiation.

Aronovitch, J., Samuni, A., Godinger, D., Greenbaum, M. and Czapski, G.

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B.V., 1986, pp. 343–345.

Other support: GSF Munich.

From the Department of Molecular Biology, School of Medicine and Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

SOD ACTIVITY *VERSUS* TOXICITY OF TERNARY COMPLEXES OF SOME COPPER COMPLEXES WITH DNA

It has been demonstrated that degradation of double-stranded DNA occurs in the presence of Cu(II) ions, 1,10-phenanthroline (OP), a reducing agent and O_2. The copper complex of 5-NO_2OP was found to be more effective than OP in cleaving DNA, while that of 2,2-bipyridyl (bipy) did not degrade DNA under the same conditions. The mechanism of the cleavage reaction was assumed to be through a site specific mechanism where the bound Cu(I) complex to DNA is oxidized by H_2O_2 to form the $OH^{\cdot}$ radical at the binding site. This mechanism explains the toxicity of the OH formed as it is formed at the binding site. Our observations indicate that no ligand dissociation takes place from bound CUL_2^{+} in the case of 5-NO_2OP but dissociation is very high in the case of bipy. In the latter case, the concentration of free CuL_2^{+} exceeds that of bound CUL_2^{+} and most of the oxidizing radicals are formed in the free solution. In the case of OP and

5-NO_2OP, most of CuL_2^+ is bound to DNA and thus the majority of the $OH^\cdot$ is formed at the binding site in a site-specific mechanism.

Czapski, G. and Goldstein, S.

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B. V., 1986, pp. 64-66.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

SUPEROXIDE DISMUTASE ACTIVITY OF SOME COPPER PHENANTHROLINE COMPLEXES AND THE MECHANISM OF THE OXIDATION OF THE VARIOUS CUPROUS COMPLEXES BY H_2O_2

A large number of copper compounds have been tested for the rate at which they react with O_2^- and some of them were found able to catalyze its dismutation with an efficiency similar to that of Cu-Zn SOD. In view of the role of CU(II) in cleaving DNA in the presence of 1,10-phenanthroline (OP), a reducing agent, and O_2^- or H_2O_2, we have studied the kinetics and mechanism of the reactions of some copper complexes of OP and substituted OP with O_2^- and H_2O_2.

Our results lead us to conclude that during the oxidation of $(OP)_2CU^+$ by H_2O_2, initially no $OH^\cdot$ is formed, but either $(OP)_2CU^{3+}$ or $(OP)_2CuH_2O_2^{3+}$ are active intermediates. We cannot distinguish between these two species because their properties are unknown and their kinetic behavior is identical.

Goldstein, S. and *Czapski, G.*

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers B. V., 1986, pp. 64-66.

From the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

IN VIVO DEGRADATION OF BACTERIAL DNA BY H_2O_2 AND O-PHENANTHROLINE

1:10-Orthophenanthroline (OP) enhances killing of *E. coli* cells by H_2O_2 and the *in vivo* fragmentation of the bacterial DNA. There is a good correlation between the bactericidal effect and the number of single and double strand breaks produced in the bacterial DNA. The results suggest that intrinsic metal ions are involved in the bactericidal effect of OP and superoxide radical is probably not an obligatory intermediate.

Aronovitch, J., Samuni, A., Godinger, D., and *Czapski, G.*

In: Rotilio, G. (editor): *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers, B. V., 1986, P. 346-348.

Other support: GSF Munich.

From the School of Medicine and Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

IMMUNOBLOTTING OF KERATIN POLYPEPTIDES EXTRACTED FROM TISSUES PRESERVED IN STANDARD HISTOLOGIC FIXATIVES

Cytoskeletal polypeptides from fresh placental tissue, tissue stored at −30°C, and tissue fixed in 10% buffered formalin, Bouin's solution, and Carnoy's solution were extracted, separated by electrophoresis, and immunoblotted using monoclonal antibodies immunoreactive with keratin polypeptides. Storage of the placental tissue at −30°C, or fixation in Carnoy's solution did not alter the extractability, migration pattern, or immunoreactivity of the keratin polypeptides. Keratin polypeptides could not be adequately demonstrated in extracts prepared from formalin- or Bouin's solution-fixed tissues. Several unmasking procedures used on tissues before extraction and on nitrocellulose blots before application of primary antibodies failed to unmask keratin polypeptides, either in Coomassie blue-stained gels or in immunoblots reacted with anti-keratin antibodies. These data indicate that Carnoy's solution is the fixative of choice for tissues in which electrophoretic and immunoblotting analyses of keratin polypeptides might be required.

Clark, R. K., and *Damjanov, I.*

The Journal of Histochemistry and Cytochemistry **34**(5):679-682, 1986.

Other support: National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

SUPPRESSION OF NONSPECIFIC BINDING OF AVIDIN-BIOTIN COMPLEX (ABC) TO PROTEINS ELECTROBLOTTED TO NITROCELLULOSE PAPER

Nitrocellulose blots of cell extracts reacted in sequence with biotinylated lectins and horseradish peroxidase-labeled avidin-biotin complex (ABC) often show considerable nonspecific staining of protein bands. Experiments were performed to determine which of the components of the ABC were responsible for this and whether or not the nature and ionic strength of the buffer used could alter this binding. Furthermore, as powdered non-fat milk has been proposed as a possible blocking agent for nonspecific binding of ABC, we sought to determine if it would adequately block that binding in our system. The initial experiments showed that nonspecific binding of ABC to proteins transferred to nitrocellulose membranes was due to the avidin component of the ABC; little, if any, binding was seen if biotin alone was incubated with these blots. The spurious binding was shown to be primarily due to the high affinity of avidin to proteins electroblotted to nitrocellulose, when incubated in low-salt buffers. Low-fat milk added to the buffer reduced overall nonspecific reactivity but produced additional artifacts in the form of bands that were not seen in other preparations. Nonspecific avidin binding to proteins transferred to nitrocellulose can therefore be effectively reduced by adding extra salt to buffers, whereas the addition of nonfat dry milk does not seem suitable for this purpose.

Clark, R. K., Tani, Y., and *Damjanov, I.*

The Journal of Histochemistry and Cytochemistry **34**(11):1509-1512, 1986.

Other support: National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine, Philadelphia.

PATHOGENESIS OF DESMOPLASIA. I. IMMUNOFLUORESCENCE IDENTIFICATION AND LOCALIZATION OF SOME STRUCTURAL PROTEINS OF LINE 1 AND LINE 10 GUINEA PIG TUMORS AND OF HEALING WOUNDS

The structural proteins of the scirrhous line 1 and the medullary line 10 bile duct carcinomas, both syngeneic in strain 2 Sewall-Wright inbred guinea pigs, were studied. Tumor structural proteins were compared with those of healing wounds. A provisional stromal matrix of cross-linked fibrin and fibronectin was initially deposited in both tumors and wounds and was subsequently replaced by granulation tissue containing collagen types I and III. The amounts of stromal fibrin-fibronectin and collagen were characteristic of each tumor: Line 1 contained significantly greater amounts than line 10. These differences were augmented when line 1 tumor rejection was prevented with cyclosporine, permitting time for stromal maturation. In tumors and wounds laminin and collagen type IV were found only in basement membranes. The findings suggest that 1) tumor desmoplasia is analogous to wound healing, 2) both processes involve replacement of an antecedent fibrin-fibronectin provisional matrix, 3) the extent of fibrin-fibronectin is characteristic of each tumor, and 4) tumor desmoplasia correlates with the amount of fibrin-fibronectin matrix deposited.

Dvorak, H. F. et al.

Journal of the National Cancer Institute **73**(5):1195-1205, November 1984.

Other support: National Cancer Institute and the National Foundation for Cancer Research

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

PATHOGENESIS OF TUMOR DESMOPLASIA. II. COLLAGENS SYNTHESIZED BY LINE 1 AND LINE 10 GUINEA PIG CARCINOMA CELLS AND BY SYNGENEIC FIBROBLASTS IN VITRO

For the investigation of the pathogenesis of desmoplasia, the capacities to synthesize collagen *in vitro* of 2 bile duct carcinomas (lines 1 and 10) of Sewall-Wright inbred strain 2 guinea pigs and of syngeneic dermal fibroblasts were studied. Line 10 cells synthesized collagen type IV as judged by sensitivity to bacterial collagenase, by immunoprecipitation, by migration of proα (IV) chains and pepsin-resistant fragments on sodium dodecyl sulfate-polyacrylamide gels, and by immunofluorescence. Line 1 cells also synthesized small amounts of collagenase-sensitive protein. Neither line 1 nor line 10 cells synthesized detectable collagen type I, III, or V. Only about 1% of [^{14}C]proline incorporated by tumor cells was found in collagenase-sensitive protein. In contrast, dermal fibroblasts synthesized 4 and 128 times as much collagenase-sensitive protein as line 10 and line 1 cells, respectively, amounting to 20% of total protein synthesized. Fibroblasts produced mostly collagen types I and III, in a ratio of 7:1, and smaller amounts of collagen type V. Thus lines 1 and 10 carcinoma cells produce primarily basement membrane collagen, whereas interstitial collagens, abundant in desmoplastic tumor stroma, are fibroblast products.

Form, D. M., VanDeWater, L., *Dvorak, H. F.*, and Smith, B. D.

Journal of the National Cancer Institute **73**(5):1207-1214, November 1984.

Other support: National Cancer Institute, Veterans Administration Merit Approved Research Program and the National Foundation for Cancer Research.

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

IMMUNOLOGICAL AND CHEMICAL PROPERTIES OF MOUSE α1-PROTEASE INHIBITORS

We previously described the isolation and purification of two similar α1-protease inhibitors from mouse plasma termed α1-PI(E) and α1-PI(T) because of their respective affinities for elastase and trypsin. Some of the biochemical and immunological properties of these proteins are reported. Both are acidic glycoproteins with PI's of 4.1-4.2. The plasma half-life of each inhibitor, determined after administration of the ^{125}I-protein, is approximately 4 h both in normal mice and in mice after induction of the acute phase reaction. The two proteins have almost identical amino acid compositions and similar CNBr peptide maps. Tryptic maps, however, are considerably different. Reverse-phase chromatography separated α1-PI(E) into three distinct isoforms, each eluting with approximately 60% acetonitrile. Under these conditions α1-PI(T) shows a single peak, clearly different from those of α1-PI(E). The three α1-PI(E) isoforms have the same molecular weights on sodium dodecyl sulfate-gel electrophoresis and the same tripeptide sequence at their N-terminus, and appear to be immunologically identical. Polyclonal, monospecific antibodies to each native inhibitor, prepared in rabbits, showed no crossreactivity when tested by functional assay or crossed immunoelectrophoresis. Interestingly, each antibody recognized epitopes on the C-terminal portion of its respective antigen. These studies confirm that α1-PI(E) and α1-PI(T), although highly similar, are products of different genes. Like human α1-PI, the two mouse inhibitors are partially inactivated by mild oxidation with chloramine-T, losing all elastase inhibitor and lesser amounts of antichymotryptic and antitryptic activity. However, unlike the human protein, neither α1-PI(E) nor α1-PI(T) was found to have methionine residue at its PI site.

Nathoo, S. A. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics **246**(1):162-174, 1986.

Other support: National Science Foundation.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

THE CO-OXIDATION OF AMMONIA TO NITRITE DURING THE AEROBIC XANTHINE OXIDASE REACTION

The xanthine oxidase reaction causes a co-oxidation of NH_3 to NO_2^-, which was inhibitable by superoxide dismutase, catalase, hydroxyl radical scavengers, or by the chelating agents, desferrioxamine or diethylene triaminepentaacetic acid. Hydroxylamine was oxidized to NO_2^- much more rapidly than was NH_3, and in this case superoxide dismutase or the chelating agents inhibited but catalase or the $HO^\cdot$ scavengers did

not. Hydrazine was not detectably oxidized to NO_2^-, and NO_2^- was not oxidized to NO_3^-, by the xanthine oxidase reaction. These results are accommodated by a reaction scheme involving (a) the metal-catalyzed production of $HO^\cdot$ from $O_2^- + H_2O_2$; (b) the oxidation of H_2N to $H_2N^\cdot$ by $OH^\cdot$; (c) the coupling of $H_2N^\cdot$ with O_2^- to yield peroxylamine, which hydrolyzes to hydroxylamine plus H_2O_2; (d) the metal-catalyzed oxidation of $HO\text{-}NH_2$ to $HO-\overset{H}{\overset{|}{N}}{}^\cdot$, which couples with O_2^- to yield $HO-\overset{H}{\overset{|}{N}}-O\overset{\cdot}{O}{}^-$, which finally dehydrates to yield NO_2^-.

Nagano, T. and *Fridovich, I.*

Archives of Biochemistry and Biophysics **241**(2):596-601, 1985.

Other support: U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

FURTHER STUDIES OF THE MECHANISM OF THE ENHANCEMENT OF NADH OXIDATION BY VANADATE

O_2^-, whether generated photochemically or introduced as a solution of KO_2 in a nonprotic solvent, caused rapid oxidation of NADH in the presence, but not in the absence, of vanadate. Superoxide dismutase inhibited this vanadate-stimulated oxidation of NADH, while catalase had no effect. This NADH oxidation appears to be a free-radical chain reaction whose average chain length was estimated to be 15 in the photochemical system. Vanadate stimulation of NADH oxidation by biological membranes can now be viewed as a sensitive indicator of O_2^- production by those membranes.

Liochev, S. and *Fridovich, I.*

Journal of Free Radicals in Biology & Medicine **1**:287-292, 1985.

Other support: National Science Foundation and U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

EFFECTS OF SALTS ON THE LETHALITY OF PARAQUAT

Escherichia coli suffered 95 to 100% lethality when exposed to 1.0 mM paraquat for 30 min at 37°C in aerobic nutrient broth medium but did not lose viability when the exposure was done in Vogel Bonner or tryptic soy yeast extract medium. Paraquat was, however, bacteriostatic in all of these media. Salts, added to the nutrient broth medium, protected against the lethality of paraquat, whereas sucrose did not. Salts of divalent cations were much more effective than salts of monovalent cations. Paraquat increases cyanide-resistant respiration by *E. coli*; salts added before, but not after, the paraquat diminished this effect. 2,4-Dinitrophenol similarly decreased the cyanide-resistant respiration when added before, but not after, the paraquat. The lethality imposed by paraquat correlated with the rate of cyanide-resistant respiration whether

this respiration was modulated by varying salt concentration at a fixed concentration of paraquat or by varying paraquat concentration at a fixed concentration of salt. We conclude that salts or 2,4-dinitrophenol interferes with the active uptake of paraquat by *E. coli* and thus prevents its lethal effect. The salt concentrations found in a number of commonly used microbiological media are sufficient to exert this effect.

Kitzler, J. and *Fridovich, I.*

Journal of Bacteriology **167**(1):346-349, 1986.

Other support: U. S. Army Research Office and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

FREE-RADICAL CHAIN OXIDATION OF 2-NITROPROPANE INITIATED AND PROPAGATED BY SUPEROXIDE

The superoxide radical $O_2^{\cdot-}$, whether produced by the xanthine/xanthine oxidase reaction or infused as KO_2, solubilized by a crown ether in dry dimethyl sulphoxide, initiated a free-radical chain oxidation of anionic 2-nitropropane. Superoxide dismutase, but not catalase, inhibited oxidation of the nitroalkane. Xanthine oxidase suffered a syncatalytic inactivation, during the co-oxidation of 2-nitropropane, which was reversed by dialysis. Cyanide exacerbated this syncatalytic inactivation and rendered it irreversible. The frequently observed oxidations of nitroalkanes by flavoenzymes now need to be re-examined to clarify the extent to which $O_2^{\cdot-}$-initiated free-radical chain oxidation contributed to the overall nitroalkane oxidation.

Kuo, C. F., and *Fridovich, I.*

Biochemical Journal **237**:505-510, 1986.

Other support: U. S. Army Research Office and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

IRREVERSIBLE INACTIVATION OF CATALASE BY 3-AMINO-1,2,4-TRIAZOLE

It is clear from the data presented and from the pertinent earlier literature that the results reported by Williams *et al.* (*Biochemical Pharmacology* **34**, 3386, 1985) must be dismissed as artifactual due to their failure to provide for a continuous supply of H_2O_2 during exposure of the catalase to 3-AT.

Darr, D., and *Fridovich, I.*

Biochemical Pharmacology **35**(20):3642, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

PHOSPHATE INHIBITION OF THE COPPER- AND ZINC-CONTAINING SUPEROXIDE DISMUTASE: A REEXAMINATION

Phosphate was reported to be an inhibitor of copper- and zinc-containing superoxide dismutase (SOD). Thus, SOD activity, in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), was decreased by approximately 50% when the assay was made 10 mM in phosphate, and the ionic strength was adjusted with sodium fluoride. The inhibitory effect of phosphate was attributed to the neutralization of the positive charge on the guanidino residue of Arg-141. We have reexamined the effects of phosphate inhibition of SOD and found that the enzyme has identical activity in phosphate or HEPES buffer when the ionic strength is adjusted with NaBr. The putative inhibitory effect of phosphate appears to have been due to fluoride inhibition of the superoxide generating system of xanthine/xanthine oxidase. We have confirmed this result by using a photochemical generation of O_2^-. Chemical modification of the lysine residues to homoarginines does not affect the activity of the enzyme and does not impart a phosphate sensitivity. Chemical modification with phenylglyoxal caused approximately 80% inactivation of the native enzyme and 90% inactivation of the *O*-methylisourea-modified enzyme. Our results suggest that phosphate does not inhibit the copper- and zinc-containing superoxide dismutase beyond the expectations of its effect on ionic strength.

Beyer, W. F., Wang, Y. and *Fridovich, I.*

Biochemistry **25**:6084-6088, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

THE VANADATE-STIMULATED OXIDATION OF NAD(P)H BY BIOMEMBRANES IS A SUPEROXIDE-INITIATED FREE RADICAL CHAIN REACTION

Rat liver microsomes catalyze a vanadate-stimulated oxidation of NAD(P)H, which is augmented by paraquat and suppressed by superoxide dismutase, but not by catalase. NADPH oxidation was a linear function of the concentration of microsomes in the absence of vanadate, but was a saturating function in the presence of vanadate. Microsomes did not catalyze a vanadate-stimulated oxidation of reduced nicotinamide mononucleotide (NMNH), but gained this ability when NADPH was also present. When the concentration of NMNH was much greater than that of NADPH a minimal average chain length could be calculated from ½ the ratio of NMNH oxidized per NADPH added. The term chain length, as used here, signifies the number of molecules of NMNH oxidized per initiating event. Chain length could be increased by increasing [vanadate] and [NMNH] and by decreasing pH. Chain lengths in excess of 30 could easily be achieved. The K_m for NADPH, arrived at from saturation of its ability to trigger NMNH oxidation by microsomes in the presence of vanadate, was 1.5 μM. Microsomes or the outer mitochondrial membrane were able to catalyze the vanadate-stimulated oxidation of NADH or NADPH but only the oxidation of NADPH was accelerated by paraquat. The inner mitochondrial membrane was able to cause the vanadate-stimulated oxidation of NAD(P)H and in this case paraquat stimulated the

oxidation of both pyridine coenzymes. Our results indicate that vanadate stimulation of NAD(P)H oxidation by biomembranes is a consequence of vanadate stimulation of NAD(P)H or NMNH oxidation by O_2^-, rather than being due to the existence of vanadate-stimulated NAD(P)H oxidases or dehydrogenases.

Liochev, S. and *Fridovich, I.*

Archives of Biochemistry and Biophysics **250**(1):139-145, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

EFFICIENCY OF PHOTOAFFINITY LABELING DNA HOMOPOLYMERS AND COPOLYMERS WITH ETHIDIUM MONOAZIDE

Photoaffinity labeling of synthetic DNAs with ethidium monoazide was studied to determine if the efficiency of adduct formation was related to DNA sequence. Equilibrium drug binding to DNA homopolymers and copolymers was quantified by phase partition techniques. The amount of drug bound to a deoxypolymer at equilibrium was then compared to the fraction of ethidium analog covalently-linked following photoactivation at the same drug/DNA input ratio. There were significant sequence-related differences in the ability of the photoaffinity probe to label DNA covalently. The efficiency of covalent-adduct formation decreased in the order poly(dG-dC)·poly(dG-dC)>poly(dG)·poly(dC)≫poly(dA-dT)·poly(dA-dT)≥poly(dA)·poly(dT). Ethidium monoazide was about 2-fold more efficient in labeling deoxyhomopolymers and deoxycopolymers composed of G-C pairs than the A-T base counterparts. In low ionic buffers (0.015 *M* Na^+), the efficiency of photoactivation decreased with increasing ethidium monoazide concentrations. However, the base sequence effect was observed over a 40-fold range of drug concentrations. Therefore, the amount of ethidium monoazide bound to a DNA site after irradiation does not appear to represent the true affinity of the drug for that site.

Dannelly, J. M., Boyce, L. and *Gaubatz, J. W.*

Photochemistry and Photobiology **43**(1):7-11, 1986.

Other support: The Hurst Foundation and the American Heart Association.

From the Department of Biochemistry, University of South Alabama, College of Medicine, Mobile.

STIMULATION OF ACTIN SYNTHESIS BY CYTOCHALASIN D IS SPECIFIC FOR THE ISOACTINS NORMALLY EXPRESSED IN MUSCLE OR NONMUSCLE CELLS

Treatment of human muscle myotube cultures with 2 μM-cytochalasin D (CD) for 6 h stimulated synthesis of both the (muscle-specific) α-actin and the (non-muscle) β and γ-actins usually expressed by these cells. In non-muscle (HEp-2) cell cultures, CD enhanced synthesis of β and γ-actin, but did not induce synthesis of α-actin, which is

not normally present in these cells. Thus, synthesis of both muscle and non-muscle actins can be increased by CD, but enhancement of actin synthesis results from increases in the isoactins usually present, rather than induction of new isotypes. Comparison of CD-treated (fused) myotube cultures with (unfused) myoblast cultures indicated that β- and γ-actin synthesis was similarly enhanced in both cultures, but that α-actin synthesis was stimulated to a greater extent in the myoblast cultures. Desmin synthesis was also stimulated in the myoblasts but not the myotubes, suggesting that the effect of CD on synthesis of these developmentally regulated cytoskeletal proteins (α-actin, desmin) might be modulated by fusion or the state of differentiation of the muscle cell.

Tannenbaum, J. and Miranda, A. F. (*Godman, G. C.*)

Journal of Cell Science **84**:253-262, 1986.

Other support: National Science Foundation, National Institutes of Health and the Muscular Dystrophy Association.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

PHENOTYPIC AND KARYOTYPIC TRANSITIONS IN THE SPONTANEOUS TRANSFORMATION OF A RAT CELL LINE

After 20–50 transfers, a rat myofibroblast line, Hmf-n, "spontaneously" transforms to an established (immortalized) line of smaller, rapidly cycling fibroblastoid cells (tHmf-f). From these 1° transformants, colonies of larger, slower growing anchorage-independent (tHmf-e) cells of epithelioid phenotype emerge. Both transformants grow in low serum and low calcium media, but the tHmf-f cells are highly tumorigenic in nude mice, have diminished substrate adhesivity, and limited anchorage independence, whereas tHmf-e are less tumorigenic, firmly substrate adherent, and markedly anchorage independent. Most tHmf-f are trisomic; most tHmf-e transformants are hypodiploid, a third are tetraploid, and all have chromosomal abnormalities, but no trisomy. Hmf-n cells have polar stress fiber arrays terminating in vinculin adhesion plaques, colinear extracellular fibronectin matrices, and linear non-coincident deposits of fodrin. Microtubules (mt) and vimentin-intermediate filaments (IF) parallel the actin cables. Stress fibers of the tHmf-f are moderately reduced, their vinculin adhesion plaques and fibronectin matrices intact; fodrin is diffuse. Mts and IFs are normal and axial. Most epithelioid tHmf-e have no stress fibers, adhesion plaques, or extracellular fibronectin; instead, dense actin microfilament meshworks are attached to plasma membrane, as is fodrin. Mt and IF are radial. Both transformed phenotypes are stable over >300 continuous passages. The differentiation-inducing agents DMSO, cyclic AMP, 5-azacytidine, and mezerein, were ineffective in normalizing shape or cytoskeleton of transformed Hmf, and butyrate was selectively toxic to 50% of tHmf-e. But hydrocortisone induced striking polarization and increase in number and alignment of stress fibers of both tHmf-f and tHmf-e. Growth, anchorage, cytoskeletal arrangements, and tumorigenic potential are not closely correlated in these stable, spontaneously transformed lines of distinct pheno- and karyotype originating from the same normal parental cell, suggesting independent acquisition of properties associated with transformation.

Brett, J. G., *Godman, G. C.*, and Miller, D. A.

TISSUE & CELL **18**(1):27-49, 1986.

Other support: National Institutes of Health and the National Cancer Institute.

From the Departments of Pathology and Human Genetics and Development, College of Physicians & Surgeons of Columbia University, New York.

CYTOCHALASIN D ALTERS THE RATE OF SYNTHESIS OF SOME HEp-2 CYTOSKELETAL PROTEINS: EXAMINATION BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

The most abundant proteins of HEp-2 cells were resolved by two-dimensional gel electrophoresis. The protein spots corresponding to several cytoskeletal proteins (vimentin, α-tubulin, β-tubulin, α-actinin, tropomyosins, and cytokeratins) were identified by comigration with protein markers or by immunological techniques.

After treatment of HEp-2 cells with 0.2 μM or 2.0 μM cytochalasin D for 20 h, radioautograms of two-dimensional gel patterns of lysates from cells pulse-labeled with [^{35}S]methionine indicated that the drug altered the rate of synthesis of some proteins. The relative rate of synthesis of the identified cytoskeletal proteins was measured. Synthesis of α-actinin, the higher-molecular-mass pair of tropomyosins and actin were similarly increased with cytochalasin D treatment, suggesting coordinate induction. Vimentin and tubulin synthesis was depressed. One cytokeratin exhibited an increase in synthesis comparable to actin, another was increased to a lesser extent and one was decreased.

Tannenbaum, J. (*Godman, G. C.*)

European Journal of Biochemistry **155**:533-542, 1986.

Other support: National Science Foundation, the Muscular Dystrophy Association and the National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

CYTOSKELETAL ORGANIZATION AFFECTS CELLULAR RESPONSES TO CYTOCHALASINS: COMPARISON OF A NORMAL LINE AND ITS TRANSFORMANT

The relationships between cytoskeletal network organization and cellular response to cytochalasin D (CD) in a normal rat fibroblast cell line (Hmf-n) and its spontaneous transformant (tHmf-e), with markedly different cytoskeletal phenotypes, were compared (using immunofluorescence, electron microscopy, and DNAse I assay for actin content). Hmf-n have prominent, polar stress fiber (SF) arrays terminating in vinculin adhesion plaques whereas tHmf-e, which are apolar, epithelioid cells with dense plasma membrane-associated actin networks, lack SF and adhesion plaques. Hmf-n exposed to CD become markedly retracted and dendritic, SF-derived actin

aggregates form large endoplasmic masses and discrete tabular aggregates at the distal ends of retraction processes. Prolonged exposure leads to recession of process, cellular rounding, and development of large cystic vacuoles. tHmf-e cells exposed to similar doses of CD display a diagnostically different response; retraction is less drastic, cells retain broad processes containing scattered actin aggregates in discrete foci often associated with plasma membrane, large tabular aggregates are never found and processes persist throughout long exposure, vacuolation is uncommon. The CD-induced microfilamentous aggregates in Hmf-n are composed of short, kinky filament fragments forming a felt-like skein, often aggregates contain a more ordered array of roughly parallel fragments, while those of tHmf-e are very short, kinky, randomly orientated filaments imparting a distinctly granular nature to the mass. Total actin content and the amount of actin associated with detergent-resistant cytoskeletons increase following CD exposure in both cell types. Throughout exposure to CD, the actin-associated contractile proteins tropomyosin, myosin, and α-actinin co-localize within the actin aggregates in both cell types. Fodrin, the protein linking cortical actin to membrane, co-localizes with actin aggregates in tHmf-e cells and most, but not all, such aggregates in Hmf-n cells, consistent with their stress fiber derivation. Vinculin is lost from the tabular aggregates at the distal ends of retraction processes in Hmf-n cells concomitant with the fragmentation and contraction of SF. The aborized processes in both cells types contain strikingly similar axial cores of bundled vimentin filaments associated with passively compressed microtubules. The characteristic CD-induced distribution of actin filament aggregates and redistribution of vimentin in these cell types also occur when cells are allowed to respread from the rounded state in the presence of CD.

Brett, J. G. and *Godman, G. C.*

TISSUE & CELL **18**(2):175-199, 1986.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

STIMULATION OF ACTIN SYNTHESIS BY CYTOCHALASIN D IS SPECIFIC FOR THE ISOACTINS NORMALLY EXPRESSED IN MUSCLE OR NON-MUSCLE CELLS

Treatment of human muscle myotube cultures with 2μM-cytochalasin D (CD) for 6 h stimulated synthesis of both the (muscle-specific) α-actin and the (non-muscle) β and γ-actins usually expressed by these cells. In non-muscle (HEp-2) cell cultures, CD enhanced synthesis of β and γ-actin, but did not induce synthesis of α-actin, which is not normally present in these cells. Thus, synthesis of both muscle and non-muscle actins can be increased by CD, but enhancement of actin synthesis results from increases in the isoactins usually present, rather than induction of new isotypes. Comparison of CD-treated (fused) myotube cultures with (unfused) myoblast cultures indicated that β and γ-actin synthesis was similarly enhanced in both cultures, but that α-actin synthesis was stimulated to a greater extent in the myoblast cultures. Desmin synthesis was also stimulated in the myoblasts but not the myotubes, suggesting that the effect of CD on synthesis of these developmentally regulated cytoskeletal proteins (α-actin, desmin) might be modulated by fusion or the state of differentiation of the muscle cell.

Tannenbaum, J. *et al.* (*Godman, G. C.*)

Journal of Cell Science **84**:253-262, 1986.

Other support: National Science Foundation, National Institutes of Health and the Muscular Dystrophy Foundation.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

CYTOCHALASIN D-INDUCED INCREASE IN ACTIN SYNTHESIS AND CONTENT IN A VARIETY OF CELL TYPES

Treatment of a variety of mesenchymal cells (normal and transformed rat fibroblasts, bovine aortic endothelial cells, rabbit smooth muscle cells), exhibiting different cytoskeletal organizations and derived from several species, with doses of cytochalasin D (CD, 2-6 μM for 20 h) sufficient to induce cytoskeletal rearrangement and altered cellular morphology results in an increase in the relative content and rate of synthesis of actin. These data extend our previous findings for HEp-2 cells to other cell types and provide further evidence for our hypothesis that the CD-induced cytoskeletal reorganization triggers stimulation of actin synthesis and the resulting increase in actin content.

Brett, J. G. and Tannenbaum, J. (*Godman, G. C.*)

Cell Biology International Reports **9**(8):723-730, August 1985.

Other support: National Science Foundation and National Institutes of Health.

From the Department of Pathology, Columbia University College of Physicians & Surgeons, New York.

EVIDENCE FOR REGULATION OF ACTIN SYNTHESIS IN CYTOCHALASIN D-TREATED HEp-2 CELLS

In HEp-2 cells treated with 0.2 or 2.0 μM cytochalasin D (CD), the relative rate of actin synthesis increased for about 12 h and then reached a plateau; this increase was suppressed by actinomycin D (AD). When CD was washed from cells which had been treated for 20 h, the elevated rate of actin synthesis declined to the control value within ca 4 h, as the actin-containing cytoskeletal components rearranged by CD recovered their normal morphology. Subsequently, actin synthesis was depressed below control values for a prolonged period; during recovery from 2 h treatment with CD, this depression was of much shorter duration. Re-addition of CD to cells after a 3 h recovery period again induced the cytoskeletal alterations characteristic of CD treatment but did not reverse the prior decline in the rate of actin synthesis. In HEp-2 cells treated with cycloheximide during exposure to CD for 20 h, the relative rate of actin synthesis measured after removal of cycloheximide was twofold higher than with CD alone and such cells exhibited a twofold slower decline in the rate of actin synthesis during recovery from CD in the continued presence of cycloheximide. These effects of cycloheximide, which resemble observations on "super-induction," suggest that actin synthesis in CD-treated and recovering HEp-2 cells may be regulated by a repressor

protein. The possibility that the proposed repressor protein is actin and that actin may thus be a feedback inhibitor of its own synthesis is discussed.

Tannenbaum, J. *et al.* (*Godman, G. C.*)

Experimental Cell Research **160**:435-448, 1985.

Other support: National Science Foundation, Muscular Dystrophy Association and National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

RED BLOOD CELLS CONTAIN A PATHWAY FOR THE DEGRADATION OF OXIDANT-DAMAGED HEMOGLOBIN THAT DOES NOT REQUIRE ATP OR UBIQUITIN

It is generally accepted that ATP is required for intracellular protein breakdown. Reticulocytes contain a soluble ATP-dependent pathway for the degradation of highly abnormal proteins and for the elimination of certain proteins during cell maturation. Reticulocytes and erythrocytes also selectively degrade proteins damaged by oxidation. When these cells were exposed to oxidants, such as phenylhydrazine or nitrite, they showed a large increase in protein breakdown. This oxidant-induced proteolysis was not inhibited in cells depleted of ATP. However, ATP depletion did prevent the degradation of pre-existent cell proteins. In reticulocyte extracts, phenylhydrazine-treated hemoglobin is also degraded rapidly by an ATP-independent process, unlike endogenous proteins and many exogenous polypeptides. This lack of an ATP requirement means that the degradation of oxidant-damaged proteins does not require ligation to ubiquitin (even though phenylhydrazine treatment does make hemoglobin a very good substrate for ubiquitin conjugation). In many respects, the pathway for breakdown of oxidant-treated hemoglobin differs from the ATP-dependent process. The latter has a much higher activation energy than the degradation of oxidized proteins. The ATP-dependent process is inhibited by hemin, 3,4-dichloroisocoumarin, diisopropylfluorophosphate and *N*-ethylmaleimide. The ATP-independent pathway is less sensitive to *N*-ethylmaleimide, hemin, and 3,4-dichloroisocoumarin and is not affected by diisopropylfluorophosphate. In addition, only the ATP-dependent proteolytic process is inactivated by dilution or incubation at 37°C in the absence of nucleotides. Reticulocytes thus contain multiple soluble systems for degrading proteins and can rapidly hydrolyze certain types of abnormal proteins by either an ATP-independent or ATP-dependent process. Erythrocytes lack the ATP-dependent process present in reticulocytes; however, erythrocytes retain the capacity to degrade oxidant-damaged hemoglobin. These two processes probably are active in the elimination of different types of abnormal proteins.

Fagan, J. M., Waxman, L. and *Goldberg, A. L.*

The Journal of Biological Chemistry **261**(13):5705-5713, May 5, 1986.

Other support: National Institute of Neurological, Communicative Diseases and Stroke.

From the Department of Physiology and Biophysics, Harvard Medical School, Boston.

THE CHEMICAL SYNTHESIS OF HIGH SPECIFIC-ACTIVITY [^{35}S] ADENOSYLHOMOCYSTEINE

The study of the family of transmethylases, critical to normal cellular function and often altered in cancer, can be facilitated by the availability of a high specific-activity *S*-adenosylhomocysteine. We report the two-step preparation of [^{35}S]adenosylhomocysteine from [^{35}S]methionine at a specific activity of 1420 Ci/mmol in an overall yield of 24% by a procedure involving demethylation of the [^{35}S]methionine to [^{35}S]homocysteine followed by condensation with 5′-chloro-5′-deoxyadenosine. The ease of the reactions, ready availability and low cost of the reagents and high specific-activity and stability of the product make the procedure an attractive one with many uses, and superior to current methodology.

Stern, P. H. and *Hoffman, R. M.*

Analytical Biochemistry **158**:408-412, 1986.

Other support: National Institutes of Health.

From the Department of Pediatrics, University of California, San Diego, La Jolla.

ORAL NICOTINE INDUCES AN ATHEROGENIC LIPOPROTEIN PROFILE

Male squirrel monkeys were used to evaluate the effect of chronic oral nicotine intake on lipoprotein composition and metabolism. Eighteen yearling monkeys were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine primates given liquid diet supplemented with nicotine at 6 mg/kg body wt day. Animals were weighed biweekly, plasma lipid, glucose, and lipoprotein parameters were measured monthly, and detailed lipoprotein composition, along with postheparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activity, was assessed after 24 months of treatment. Although nicotine had no effect on plasma triglyceride or high density lipoproteins (HDL), the alkaloid caused a significant increase in plasma glucose, cholesterol, and low density lipoprotein (LDL) cholesterol plus protein while simultaneously reducing the HDL cholesterol/plasma cholesterol ratio and animal body weight. Levels of LDL precursors, very low density (VLDL) and intermediate density (IDL) lipoproteins, were also lower in nicotine-treated primates while total postheparin lipase (LPL + HTGL) activity was significantly elevated. Our data indicate that long-term consumption of oral nicotine induces an atherogenic lipoprotein profile (↑ LDL, ↓ HDL/total cholesterol ratio) by enhancing lipolytic conversion of VLDL to LDL. These results have important health implications for humans who use smokeless tobacco products or chew nicotine gum for prolonged periods.

Cluette-Brown, J., Mulligan, J., Doyle, K., Hagan, S., Osmolski, T., and *Hojnacki, J.*

Proceedings of the Society for Experimental Biology and Medicine **182**(3):409-413, 1986.

From the Department of Biological Sciences, Graduate Biochemistry Program, University of Lowell, Lowell, MA.

ORAL NICOTINE IMPAIRS CLEARANCE OF PLASMA LOW DENSITY LIPOPROTEINS

The effect of chronic oral nicotine intake on plasma low density lipoprotein (LDL) clearance, lipid transfer protein, and lecithin:cholesterol acyltransferase (LCAT) was examined in male atherosclerosis susceptible squirrel monkeys. Eighteen yearling primates were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine monkeys given liquid diet supplemented with nicotine at 6 mg/kg body wt/day for a two-year period. Averaged over 24 months of treatment, animals in the Nicotine group had significantly higher levels of plasma and LDL cholesterol compared to Controls while plasma LCAT activity was similar for both groups. Following simultaneous injection of ^{3}H LDL and ^{14}C high density lipoprotein (HDL) cholesterol ester (CE), removal of the latter was not altered by oral nicotine while plasma clearance of ^{3}H LDL was dramatically delayed in Nicotine monkeys. Transfer of ^{14}C HDL CE to very low density lipoprotein (VLDL)-LDL particles was greatly accelerated in the Nicotine group *vs* Controls while the reciprocal movement of ^{3}H LDL CE to HDL was only higher in experimental animals at two time points following injection of the isotopes. Results from this study provide evidence that one major detrimental effect of long-term oral nicotine use is an increase in the circulating pool of atherogenic LDL which is due to: 1) accelerated transfer of lipid from HDL; and 2) impaired clearance of LDL from the plasma compartment. Diminished removal of LDL is of particular importance because an extended residence time of these particles in circulation would increase the likelihood of their deposition in the arterial wall.

Hojnacki, J. et al.

Proceedings of the Society for Experimental Biology and Medicine **182**(3):414-418, 1986.

From the Department of Biological Sciences, Graduate Biochemistry Program, University of Lowell, Lowell, MA.

PHOSPHORYLATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR REGULATES ITS RATE OF DESENSITIZATION

Recent studies have provided evidence for a role of protein phosphorylation in the regulation of the function of various potassium and calcium channels. As these ion channels have not yet been isolated and characterized, it has not been possible to determine whether phosphorylation of the ion channels themselves alters their properties or whether some indirect mechanism is involved. In contrast, the nicotinic acetylcholine receptor, a neurotransmitter-dependent ion channel, has been extensively characterized biochemically and has been shown to be directly phosphorylated. The phosphorylation of this receptor is catalysed by at least three different protein kinases (cyclic AMP-dependent protein kinase, protein kinase C and a tyrosine-specific protein kinase) on seven different phosphorylation sites. However, the functional significance of phosphorylation of the receptor has been unclear. We have now examined the functional effects of phosphorylation of the nicotinic acetylcholine receptor by cAMP-dependent protein kinase. We investigated the ion transport properties of the purified and reconstituted acetylcholine receptor before and after phosphorylation. We report here that phosphorylation of the nicotinic acetylcholine receptor on the γ- and δ--

subunits by cAMP-dependent protein kinase increases the rate of the rapid desensitization of the receptor, a process by which the receptor is inactivated in the presence of acetylcholine (ACh). These results provide the first direct evidence that phosphorylation of an ion channel protein modulates its function and suggest that phosphorylation of postsynaptic receptors in general may play an important role in synaptic plasticity.

Huganir, R. L. et al.

Nature **321**(6072):774-776, 1986.

Other support: National Science Foundation.

From the Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, and Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY.

INTERACTIONS OF POLYCLONAL ANTI-*ELECTROPHORUS* NICOTINIC RECEPTOR ANTISERA WITH *TORPEDO* NICOTINIC RECEPTOR

Polyclonal antisera raised against solubilized and purified nicotinic acetylcholine receptor from *Electrophorus* electroplax and a polyclonal anti-α-bungarotoxin antiserum have been studied by the use of four different radioimmunoassay protocols. The results indicate unique sensitivities of different assay techniques in analysis of antibody-antigen interactions, and serve as a model for immunological study of other integral membrane proteins.

Lukas, R. J.

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Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, Inc., and the Men's and Women's Boards of the Barrow Neurological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

INTERACTIONS OF ANTINICOTINIC ACETYLCHOLINE RECEPTOR ANTIBODIES WITH RAT BRAIN AND MUSCLE ANTIGENIC DETERMINANTS

Studies were performed to determine whether antibodies prepared against nicotinic acetylcholine receptors (nAcChoR) from electric tissue are reactive toward nAcChoR-like antigenic determinants in rat brain. Reference experiments involved the use of *Torpedo* electroplax and rat innervated muscle as tissue controls and an anti-α-bungarotoxin antiserum as a probe for curaremimetic neurotoxin binding sites. As evidenced by their ability to inhibit immunoprecipitation of *Torpedo* nAcChoR, brain or muscle membranes specifically interact with polyclonal antisera raised against *Electrophorus* electroplax nAcChoR. When the extent of polyclonal anti-nAcChoR antibody binding to muscle membranes is measured by protein A binding protocols, receptor-like antigenic determinants and toxin binding sites are found to be present in approximately equal quantities. In contrast, nAcChoR-like antigenic determinants on rat brain membranes are present at concentrations in excess of those of toxin binding

sites. The results are consistent with the earlier observation that some antibodies prepared against nAcChoR from peripheral tissues recognize rat brain high-affinity α-bungarotoxin binding sites. The results also suggest the existence of nAcChoR-like entities in brain that do not bind toxin with a high affinity.

Lukas, R. J.

Cellular and Molecular Neurobiology **6**(3):281-291, 1986.

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From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

CHARACTERIZATION OF CURAREMIMETIC NEUROTOXIN BINDING SITES ON MEMBRANE FRACTIONS DERIVED FROM THE HUMAN MEDULLOBLASTOMA CLONAL LINE, TE671

Studies were conducted on curaremimetic neurotoxin binding to the nicotinic acetylcholine receptor present on membrane fractions derived from the human medulloblastoma clonal line, TE671. High-affinity binding sites (K_D = 2 n*M* for 1-h incubation at 20°C) and low-affinity binding sites (K_D = 40 n*M*) for ^{125}I-labeled α-bungarotoxin are present in equal quantities (60 fmol/mg membrane protein). The kinetically determined dissociation constant for high-affinity binding of toxin is 0.56 n*M* (k_1 = $6.3 \cdot 10^{-3}$ min^{-1} nM^{-1}; k_{-1} = $3.5 \cdot 10^{-3}$ min^{-1}) at 20°C. Nicotine, *d*-tubocurarine, and acetylcholine are among the most effective inhibitors of high-affinity toxin binding. The quantity of toxin binding sites and their affinity for cholinergic agonists are sensitive to reduction, alkylation, and/or oxidation of membrane sulfhydryl residues. High-affinity toxin binding sites that have been subjected to reaction with the sulfhydryl reagent dithiothreitol are irreversibly blocked by the nicotinic receptor affinity reagent bromoacetylcholine. High-affinity toxin binding is inhibited in the presence of either of two polyclonal antisera or a monoclonal antibody raised against nicotinic acetylcholine receptors from fish electric tissue. Taken together, these results indicate that curaremimetic neurotoxin binding sites on membrane fractions of the TE671 cell line share some properties with nicotinic acetylcholine receptors of peripheral origin and with toxin binding sites on other neuronal tissues.

Lukas, R. J.

Journal of Neurochemistry **46**(6):1936-1941, 1986.

Other support: Men's and Women's Boards of the Barrow Neurological Foundation, Epi-Hab Phoenix and the National Institutes of Health.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

CHARACTERIZATION OF CURAREMIMETIC NEUROTOXIN BINDING SITES ON CELLULAR MEMBRANE FRAGMENTS DERIVED FROM THE RAT PHEOCHROMOCYTOMA PC12

Studies were conducted on the properties of ^{125}I-labeled α-bungarotoxin binding

sites on cellular membrane fragments derived from the PC12 rat pheochromocytoma. Two classes of specific toxin binding sites are present at approximately equal densities (50 fmol/mg of membrane protein) and are characterized by apparent dissociation constants of 3 and 60 n*M*. Nicotine and *d*-tubocurarine are among the most potent inhibitors of high-affinity toxin binding. The affinity of high-affinity toxin binding sites for nicotinic cholinergic agonists is reversibly or irreversibly decreased, respectively, on treatment with dithiothreitol or dithiothreitol and *N*-ethylmaleimide. The nicotinic receptor affinity reagent bromoacetylcholine irreversibly blocks high-affinity toxin binding to PC12 cell membranes that have been treated with dithiothreitol. Two polyclonal antisera raised against the nicotinic acetylcholine receptor from *Electrophorus electricus* inhibit high-affinity toxin binding. These detailed studies confirm that curaremimetic neurotoxin binding sites on the PC12 cell line are comparable to toxin binding sites from neural tissues and to nicotinic acetylcholine receptors from the periphery. Because toxin binding sites are recognized by anti-nicotinic receptor antibodies, the possibility remains that they are functionally analogous to nicotinic receptors.

Lukas, R. J.

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Other support: Men's and Women's Boards of the Barrow Neurological Foundation, Epi-Hab Phoenix and the National Institutes of Health.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

INTERACTIONS OF ANTI-NICOTINIC ACETYLCHOLINE RECEPTOR ANTIBODIES AT α-BUNGAROTOXIN BINDING SITES ACROSS SPECIES AND TISSUES

Two antisera prepared against the nicotinic acetylcholine receptor (nAcChoR) from *Electrophorus* exhibit comparable ability to inhibit high-affinity α-bungarotoxin binding to membrane fractions from rat brain or muscle, PC12 or TE671 cells, or *Torpedo* electric tissue. Only one of several monoclonal antibodies raised against nAcChoR from *Torpedo* inhibits toxin binding to membranes from rat brain or muscle or TE671 cells, but is considerably more potent as an inhibitor of toxin binding to *Torpedo* nAcChoR. These results indicate that some antibodies prepared against nAcChoR from electric tissue recognize epitopes near the high-affinity toxin binding sites. Some of these toxin binding site epitopes are preserved across species and tissue. The positive outcome of this study supports the continued use of toxin as a probe for at least a subset of mammalian neuronal nAcChoR.

Lukas, R. J.

Molecular Brain Research **1**:119-125, 1986.

Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, and the Men's and Women's Boards of the Barrow Neurological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

ISOLATION, GROWTH REQUIREMENTS, CLONING, PROSTACYCLIN PRODUCTION AND LIFE-SPAN OF HUMAN ADULT ENDOTHELIAL CELLS IN LOW SERUM CULTURE MEDIUM

Endothelial cells from autopsy and biopsy specimens from a variety of adult human vascular tissue were harvested by collagenase treatment and gentle swabbing of the lumenal surface. Nutrient medium MCDB 107 containing a partially purified brain-derived growth factor (5 μg/ml), epidermal growth factor (10 ng/ml) and only 2% (v/v) fetal bovine serum supported clonal and long-term serial culture (17.6 to 26.1 cumulative population doublings) of endothelial cells from vena cava, thoracic aorta and tibial arteries at a 70% rate of success. Cumulative doublings of the cell population from eight cultures were inversely proportional to age of donor of the vascular tissue from which cells were isolated. Heparin had an enhancing effect on cell growth that varied with cell strain. Prostacyclin production of human adult endothelial cell cultures was stimulated by arachidonate and thrombin by 17- to 20-fold, and 2- to 3-fold, respectively. Endogenous and stimulated rates of prostacyclin production by human adult endothelial cells were 2 to 3 times that of human adult smooth muscle cells and 20 to 30 times that of human fibroblasts.

Hoshi, H., and *McKeehan, W. L.*

In vitro Cellular & Developmental Biology **22**(1):51-56, 1986.

Other support: U. S. Public Health Service.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

CHARACTERIZATION OF MULTIPLE FORMS OF PROSTATROPIN (PROSTATE EPITHELIAL CELL GROWTH FACTOR) FROM BOVINE BRAIN

Two molecular forms of prostatropin distributed among five chromatographic peaks have been isolated from bovine brain by heparin-Sepharose affinity and reverse phase high performance liquid chromatography. One form has an apparent molecular weight of 16000 and an amino terminal sequence of asn-tyr-lys-lys-pro-lys-leu-leu-tyr-x-ser-asn-gly. The other form has an apparent molecular weight of 18000 and a blocked amino terminus. Both forms are similar in amino acid composition. The sequence of a proteolytic fragment from the blocked form overlaps the NH_2-terminal sequence of the unblocked form, therefore, the smaller form may be derived from the larger form through proteolytic processing. Both forms contain regions identical in sequence to brain-derived, heparin-binding growth factors that have been isolated on the basis of mitogenic activity for fibroblasts and endothelial cells. Both forms of prostatropin exhibit potent mitogenic activity for normal and tumor prostate epithelial cells.

Crabb, J. W., Armes, L. G., Johnson, C. M., and *McKeehan, W. L.*

Biochemical and Biophysical Research Communications **136**(3):1155-1161, May 14, 1986.

Other support: National Cancer Institute.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

TWO APPARENT HUMAN ENDOTHELIAL CELL GROWTH FACTORS FROM HUMAN HEPATOMA CELLS ARE TUMOR-ASSOCIATED PROTEINASE INHIBITORS

Two polypeptides from secretory products of human hepatoma cells were isolated and characterized on the basis of their stimulation of maintenance and growth of human endothelial cells in serum-free cell culture. Both factors were purified to homogeneity by a combination of reverse-phase, ion exchange, and molecular filtration high performance liquid chromatography. One factor (endothelial cell growth factor (ECGF-2a)) had $M_r \sim 6,500$ and pI near 6. The second (ECGF-2b) had $M_r = 27,000$ and a pI below 4.0. Both ECGF-2a and ECGF-2b exhibited single NH_2-terminal sequences. The first 25 NH_2-terminal residues of ECGF-2a and the first 49 residues of ECGF-2b were determined by gas-phase microsequencing. All clearly determined residues of ECGF-2a were identical with human pancreatic secretory trypsin inhibitor. All assignable residues of ECGF-2b were identical with urinary glycoprotein proteinase inhibitor (HI-30/EDC1). Both proteins are absent or at low levels in normal plasma and urine, but appear during acute inflammatory disease and cancer. Amino acid composition of ECGF-2a and ECGF-2b was also similar to human pancreatic secretory inhibitor and HI-30/EDC1, respectively. Both ECGF-2a and ECGF-2b inhibited bovine pancreatic trypsin (2 μg/ml) by 50% at 750 ng/ml. ECGF-2a and ECGF-2b stimulated endothelial cell number at a half-maximal dose of 50 ng/ml (8 nM) and 80 to 130 ng/ml (5 to 9 nM) protein, respectively. When assayed under identical conditions, no effect of either factor on human smooth muscle cells, human hepatoma cells, or human, rat, and mouse fibroblasts could be detected.

McKeehan, W. L. et al.

The Journal of Biological Chemistry **216**(12):5378-5383, 1986.

Other support: United States Public Health Service.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

COMPLETE PRIMARY STRUCTURE OF PROSTATROPIN, A PROSTATE EPITHELIAL CELL GROWTH FACTOR

Bovine brain prostatropin is a potent and essential mitogen for prostate epithelial cell growth. The major form of prostatropin contains 154 amino acid residues in a single amino terminally blocked chain corresponding to a molecular weight of 17400. The amino acid sequence of the 150 carboxy-terminal residues of prostatropin was derived by Edman degradation of overlapping peptides primarily generated by cleavage at lysyl and glutamyl residues. Analysis of the amino-terminal tetradecapeptide by fast atom bombardment mass spectrometry identified the blocking group as an acetyl moiety, and tandem mass spectrometry provided the sequence of the first 12 residues. Prostatropin residues 15–154 contain the sequence of bovine brain polypeptides recently described as acidic fibroblast growth factor and class I heparin-binding growth factor. The sequence of the first 25 residues of prostatropin is acetyl-Ala-(Gly, Glu)-Glu-Thr-Thr-Thr-Phe-Thr-Ala-Leu-Thr-Glu-Lys-Phe-Asn-Leu-Pro-Leu-Gly-Asn-Tyr-Lys-Lys-Pro. Reduced and carboxymethylated prostatropin exhibits mitogenic activity, suggesting that disulfide bonds among cysteine residues 30, 61, and 97 are not functionally essential. These results demonstrate by rigorous structural analysis that the brain-derived polypeptide previously described only as a mesenchymal and neuro-

ectodermal cell mitogen is also an epithelial cell growth factor that may be involved in support of prostate hyperplasia and adenocarcinoma.

Crabb, J. W., Armes, L. G., Carr, S. A., and *McKeehan, W. L.*

Biochemistry **25**:4988-4993, 1986.

Other support: National Institutes of Health.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

ROLE OF LIPOPROTEINS IN GROWTH OF HUMAN ADULT ARTERIAL ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN LOW LIPOPROTEIN-DEFICIENT SERUM

Recently improved culture conditions for human adult arterial endothelial and smooth muscle cells from a wide variety of donors have been used to study the effects of lipoproteins on proliferation of both cell types in low serum culture medium. Optimal growth of endothelial and smooth muscle cells in an optimal nutrient medium (MCDB 107) containing epidermal growth factor, a partially purified fraction from bovine brain, and 1% (v/v) lipoprotein-deficient serum was dependent on either high- or low-density lipoprotein. High- and low-density lipoprotein stimulated cell growth by three- and five-fold, respectively, over a 6-day period. Optimal stimulation of both endothelial and smooth muscle cell growth occurred between 20 and 60 μg/ml of high- and low-density lipoproteins, respectively. No correlation between the activation of 3-hydroxyl-3-methylglutaryl coenzyme A reductase activity and lipoprotein-stimulated cell proliferation was observed. Lipid-free total apolipoproteins or apolipoprotein C peptides from high-density lipoprotein were partially effective and together with oleic acid effectively replaced native high-density lipoprotein for the support of endothelial cell growth. In contrast, apolipoproteins or apolipoprotein C peptides from high-density lipoprotein alone or with oleic acid had no effect on smooth muscle cell proliferation. The results suggest a functional role of high- and low-density lipoproteins and apolipoproteins in the proliferation of human adult endothelial and smooth muscle cells.

Chen, J.-K., Hoshi, H., McClure, D. B., and *McKeehan, W. L.*

Journal of Cellular Physiology **129**:207-214, 1986.

Other support: National Cancer Institute and the National Institutes of Health.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

EFFECT OF CYANIDE ON NITROVASODILATOR-INDUCED RELAXATION, CYCLIC GMP ACCUMULATION AND GUANYLATE CYCLASE ACTIVATION IN RAT AORTA

The effects of sodium cyanide on relaxation, increases in cyclic GMP accumulation and guanylate cyclase activation induced by sodium nitroprusside and other nitrovasodilators were examined in rat thoracic aorta. Cyanide abolished nitroprusside-induced relaxation and the associated increase in cyclic GMP levels. Basal levels of cyclic GMP and cyclic AMP were also depressed. Reversal of nitroprusside-induced relaxation by cyanide was independent of the tissue level of cyclic GMP prior to

addition of cyanide. Incubation of nitroprusside with cyanide prior to addition to aortic strips did not alter the relaxant effect of nitroprusside. Sodium azide-, hydroxylamine-, N-methyl-N'-nitro-N-nitrosoguanide-, nitroglycerin- and acetylcholine-induced relaxations and increased levels of cyclic GMP were also inhibited by cyanide. Relaxations induced by nitric oxide were also inhibited by cyanide, although the relaxation with the low concentration of nitric oxide employed was not accompanied by detectable increases in cyclic GMP. Relaxation to 8-bromo-cyclic GMP was essentially unaltered by cyanide; however, isoproterenol-induced relaxation was inhibited. Guanylate cyclase in soluble and particulate fractions of aorta homogenates was activated by nitroprusside and the activation was prevented by cyanide. The present results suggest that cyanide inhibits nitrovasodilator-induced relaxation through inhibition of guanylate cyclase activation; however, cyanide may also have nonspecific effects which inhibit relaxation.

Rapoport, R. M. and *Murad, F.*

European Journal of Pharmacology **104**:61-70, 1984.

Other support: National Institutes of Health, Veterans Administration, and a National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

SPECIFIC ATRIAL NATRIURETIC FACTOR RECEPTORS MEDIATE INCREASED CYCLIC GMP ACCUMULATION IN CULTURED BOVINE AORTIC ENDOTHELIAL AND SMOOTH MUSCLE CELLS

ANF is a newly discovered group of small peptides that exhibit potent vasodilatory activity.[1-5] In order to acquire a better understanding of the mechanism of action of ANF on vascular tissue, we used ^{125}I-ANF to identify receptors on cultured bovine aortic endothelial and smooth muscle cells. Scatchard analysis indicated that BASM cells contain a single class of high affinity binding sites with a K_D of 0.9 nM. BASM cells had 111,000 sites/cell. Similar results were recently reported by other investigators using rat[3] and bovine[4] aortic smooth muscle cells. We also have found that bovine aortic endothelial cells contain higher affinity (K_D = 0.1 nM) binding sites for ^{125}I-ANF (8–33), but a fewer number of receptors (16,000 sites/cell).[2] Five different atrial natriuretic peptides inhibited ^{125}I-ANF binding and stimulated cyclic GMP accumulation with the same order of potency when BASM or endothelial cells were examined. However, atriopeptin I was more effective at inhibiting ^{125}I-ANF binding than increasing cyclic GMP. While the K_i for atriopeptin I was about 6-fold higher than the K_i for ANF (8–33), it took at least a 100 times higher concentration for atriopeptin I to elicit the same magnitude of cyclic GMP stimulation in BASM cells. The disparity between the ability of atriopeptin I to inhibit ^{125}I-ANF binding and stimulate cyclic GMP was even more marked in endothelial cells.[2] In these cells the K_i for antriopeptin I to inhibit ^{125}I-ANF binding was 6-fold higher than the K_i for ANF (8–33), while the EC_{50} for atriopeptin I to increase cyclic GMP levels was at least 1500-fold greater. These results demonstrate that atriopeptin I is able to bind to ANF receptors effectively in both endothelial and smooth muscle cells, but is a weak stimulator of cyclic GMP accumulation.

Leitman, D. C., Waldman, S. A., Rapoport, R. M., and *Murad, F.*

Transactions of the Association of American Physicians XCVIII, pps. 243-252, 1985.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

AMINOPHYLLINE POTENTIATES SODIUM NITROPRUSSIDE-INDUCED HYPOTENSION IN THE DOG

The biochemical mechanisms by which nitroso-vasodilators cause smooth muscle relaxation remain controversial. One theory states that the effects of nitroso-vasodilators are mediated by increased intracellular levels of cyclic GMP due to activation of guanylate cyclase. To test this hypothesis, the authors examined the effects of sodium nitroprusside (SNP) in anesthetized dogs with and without pretreatment with the phosphodiesterase inhibitor aminophylline. Aminophylline pretreatment resulted in a 2.8-fold potentiation of the hypotensive effects of a continuous infusion of SNP. Potentiation also was seen for the effects of SNP on stroke volume, heart rate, and plasma cyclic GMP levels. These results support the hypothesis that nitroso-vasodilators exert their effects via guanylate cyclase activation. The authors advise caution when vasodilator therapy with agents such as SNP, nitroglycerin, or hydralazine is instituted in patients receiving aminophylline and when aminophylline is either instituted in patients receiving aminophylline and when aminophylline is either instituted or discontinued in patients on vasodilator therapy.

Pearl, R. G., Rosenthal, M. H., *Murad, F.*, and Ashton, J. P. A.

Anesthesiology **61**(6):712-715, 1984.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Anesthesia and Medicine, Stanford University Medical Center, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

ENDOTHELIUM-DEPENDENT AND NITROVASODILATOR-INDUCED ACTIVATION OF CYCLIC GMP-DEPENDENT PROTEIN KINASE IN RAT AORTA

Cyclic GMP-dependent protein kinase (cyclic GMP-kinase) activity in isolated strips of rat aorta was measured in the absence and presence of exogenous cyclic GMP (2 μM) and expressed as a ratio. This activity ratio represented an estimate of the endogenous activation state of the enzyme. Acetylcholine (10 μM), an endothelium-dependent vasodilator, increased the activity ratio from a control value of 0.42 to 0.71 in aorta with endothelium intact. With endothelium removed, acetylcholine had no effect on cyclic GMP-kinase activity. The nitrovasodilator sodium nitroprusside (50 nM) increased activity ratios in aorta both with (0.42 to 0.54) and without (0.29 to 0.40) endothelium. Since activity ratios were higher in aortas with an intact endothelium, a tonic influence of the endothelium on aorta cyclic GMP-kinase is suggested. The vasodilator isoproterenol (3μM) had no effect on cyclic GMP-kinase activity ratios. The increases in cyclic GMP-kinase activity caused by sodium nitroprusside

and acetylcholine were preserved when aortas were homogenized in buffer containing 3 mg/ml charcoal. Thus, most of the cyclic GMP-kinase activation occurred in the intact tissue and not because of endogenous cyclic nucleotides present during homogenization or assay. The increases in the activity ratio to sodium nitroprusside and acetylcholine correlate with increases in cyclic GMP concentration and with smooth muscle relaxation. It is concluded that cyclic GMP-kinase in rat aorta is activated by acetylcholine in an endothelium-dependent manner and by sodium nitroprusside in an endothelium-independent manner. These data are consistent with the hypothesis that cyclic GMP mediates relaxation of vascular smooth muscle to acetylcholine and sodium nitroprusside by activating cyclic GMP-kinase and consequent protein phosphorylation. The data further illustrate the importance of endothelial cells in vascular responses to acetylcholine.

Fiscus, R. R., Rapoport, R. M., and *Murad, F.*

Journal of Cyclic Nucleotide and Protein Phosphorylation Research 9(6):415-425, 1983-84.

Other support: National Institutes of Health, Veterans Administration and the American Heart Association, California Affiliate.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

EFFECTS OF THE D AND L STEREOISOMERS OF ISOIDIDE DINITRATE ON RELAXATION AND CYCLIC GMP ACCUMALATION IN RAT AORTA AND COMPARISON TO GLYCERYL TRINITRATE

The effects of the D- and L-stereoisomers of isoidide dinitrate on relaxation and cyclic GMP accumulation were compared in isolated rat aorta. Although both isomers were equally efficacious as vasorelaxants, the D-isomer was approximately ten times more potent that the L-isomer and about one-tenth the potency of glyceryl trinitrate. The median effective concentration of the D- and L-isomers for relaxation was 0.45 ± 0.06 μM and 6.7 ± 0.7 μM, respectively. The time-course of relaxation and elevation of cyclic GMP were closely correlated; the maximal relaxation and cyclic GMP accumulation occurred at 2 min. Consistent with the potency difference for relaxation, glyceryl trinitrate was ten times more potent than D-isoidide dinitrate, which was ten times more potent than the L-isomer with regard to the elevation of cyclic GMP levels. It is concluded that stereospecificity plays a role in organic nitrate-induced elevation of cyclic GMP and vasodilation.

Bennett, B. M., Hayward, L. D., and *Murad, F.*

Journal of Applied Cardiology 1:203-209, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA; and the Department of Chemistry, University of British Columbia, Vancouver, Canada.

SOLUBLE GUANYLATE CYCLASE FROM RAT LUNG EXISTS AS A HETERODIMER

The soluble form of guanylate cyclase (EC 4.6.1.2) from rat lung has been purified to homogeneity by one-step immunoaffinity chromatographic procedure. The purified soluble guanylate cyclase has specific activities of 432 and 49.1 nmol of cyclic GMP formed per min/mg protein with manganese and magnesium ions as a cofactor, respectively. This represents a purification of approximately 2,000-fold with a 50% recovery. The native enzyme has a molecular weight of 150,000 and a Stokes radius of 4.8 nm as determined on Spherogel TSK-G3000SW gel permeation chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis results in two protein-staining bands with molecular weights of 82,000 and 70,000. The purified soluble guanylate cyclase was also subjected to native polyacrylamide gel electrophoresis, isoelectric focusing electrophoresis, ion exchange chromatography, and GTP-agarose affinity chromatography. These additional purification procedures confirmed the presence of a single protein peak coincident with enzyme activity. The two subunits separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis were shown to have different primary structures by immunoblotting with monoclonal and polyclonal antibodies prepared against purified soluble guanylate cyclase and by peptide mapping with papain or *Staphylococcus aureus* V8 protease treatment. The data demonstrate that soluble guanylate cyclase purified from rat lung is a heterodimer composed of 82,000- and 70,000-dalton subunits with different primary structures.

Kamisaki, Y., Saheki, S., Nakane, M., Palmieri, J. A., Kuno, T., Chang, B. Y., Waldman, S. A. and *Murad, F.*

The Journal of Biological Chemistry **261**(16):7236-7241, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

INTESTINAL RECEPTOR FOR HEAT-STABLE ENTEROTOXIN OF *ESCHERICHIA COLI* IS TIGHTLY COUPLED TO A NOVEL FORM OF PARTICULATE GUANYLATE CYCLASE

A novel form of particulate guanylate cyclase tightly coupled by cytoskeletal components to receptors for heat-stable enterotoxin (ST) produced by *Escherichia coli* can be found in membranes from rat intestinal mucosa. Intestinal particulate guanylate cyclase was resistant to solubilization with detergent alone, with only 30% of the total enzyme activity being extracted with Lubrol-PX. Under similar conditions, 70% of this enzyme was solubilized from rat lung membranes. The addition of high concentrations of sodium chloride to the extraction buffer resulted in greater solubilization of particulate guanylate cyclase from intestinal membranes. Although extraction of intestinal membranes with detergent and salt resulted in greater solubilization of guanylate cyclase, a small fraction of the enzyme activity remained associated with the particulate fraction. This activity was completely resistant to solubilization with a variety of

detergents and chaotropes. Particulate guanylate cyclase and the ST receptor solubilized by detergent retained their abilities to produce cyclic GMP and bind ST, respectively. However, ST failed to activate particulate guanylate cyclase in detergent extracts. In contrast, guanylate cyclase resistant to solubilization remained functional and coupled to the ST receptor since enzyme activation by ST was unaffected by various extraction procedures. The possibility that the ST receptor and particulate guanylate cyclase were the same molecule was explored. ST binding and cyclic GMP production were separated by affinity chromatography on GTP-agarose. Similarly, guanylate cyclase migrated as a 300,000-dalton protein, while the ST receptor migrated as a 240,000-dalton protein on gel filtration chromatography. Also, thiol-reactive agents such as cystamine and *N*-ethylmaleimide inhibited guanylate cyclase activation by ST, with no effect on receptor binding of ST. These data suggest that guanylate cyclase and the ST receptor are independent proteins coupled by cytoskeletal components in membranes of intestinal mucosa.

Waldman, S. A., Kuno, T., Kamisaki, Y., Chang, L. Y., Gariepy, J., O'Hanley, P., Schoolnik, G., and *Murad, F.*

Infection and Immunity **51**(1):320-326, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine, Pharmacology, and Microbiology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CYCLIC GUANOSINE MONOPHOSPHATE AS A MEDIATOR OF VASODILATION

Although cyclic guanosine monophosphate (GMP) was first described in biological samples more than two decades ago, its role in some physiological processes has only become apparent in the past few years. This relatively slow development is probably attributable to the low concentrations of the nucleotide in tissues, the complex and insensitive methods available during the early studies, and the biases many investigators had regarding its possible functions. The latter was undoubtedly influenced by the many similarities of the cyclic GMP system with that of cyclic AMP and the attention cyclic AMP has received during this period. While analogies and similarities between these two cyclic nucleotide systems do exist, the cyclic GMP system presents more complexities due to the existence of several isoenzymes responsible for its synthesis. It is known that the conversion of guanosine triphosphate (GTP) to cyclic GMP is catalyzed by at least two isoenzyme forms of guanylate cyclase. The kinetic, physicochemical, and antigenic properties of the cytosolic and membrane-associated isoenzymes are quite different. The relative abundance of the soluble and particulate enzyme is variable in different tissues and species. While intestinal mucosa and retina possess predominately the particulate isoenzyme and platelets contain the soluble isoenzyme, most tissues such as vascular smooth muscle have both isoenzymes. Furthermore, the regulation of each of these isoenzymes is quite different. The soluble enzyme appears unique in that it can be activated by reactive free radicals such as nitric oxide,

and probably hydroxyl free radical and some porphyrins. On the other hand, the particulate isoenzyme can be activated with agents such as *Escherichia coli* heat-stable enterotoxin, atriopeptins, and hemin. Cations, thiols, other redox agents, and detergents also have complex effects on the activity of both isoenzymes.

Murad, F.

Journal of Clinical Investigation **78**:1-5, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CHARACTERIZATION OF THE RECEPTOR FOR HEAT-STABLE ENTEROTOXIN FROM *ESCHERICHIA COLI* IN RAT INTESTINE

The receptor for the heat-stable enterotoxin (ST) from *Escherichia coli* was solubilized with Lubrol-PX from rat intestinal brush-border membranes and characterized. The binding kinetics and analog specificity of the solubilized receptor were virtually identical to those obtained with the membrane-bound receptor. Furthermore, the regulation of the receptor's affinity by cations was also maintained after solubilization, indicating a conservation of the toxin-binding site after removal of the receptor from its membrane environment. Gel filtration and sucrose density gradient sedimentation studies gave a Stokes radius of 5.5 nm and a sedimentation coefficient of 7.0 S for the solubilized receptor. The isoelectric point of the receptor was determined as 5.5 using Sephadex isoelectric focusing electrophoresis. In all of these separation techniques, the ST receptor showed a single peak of activity that was clearly separated from that of guanylate cyclase. When ^{125}I-ST was cross-linked to brush-border membranes with disuccinimidyl suberate, the affinity-labeled receptor solubilized with 0.1% Lubrol-PX eluted at a similar position as the native receptor on gel filtration chromatography. Analysis of the affinity-labeled receptor by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of reducing agent and by autoradiography revealed the presence of three specifically labeled polypeptides with apparent molecular weights of 80,000, 68,000, and 60,000. These results suggest that the ST receptor is solubilized by Lubrol-PX in an active form with preservation of its regulation by cations. Also, the ST receptor is separable from particulate guanylate cyclase indicating that the receptor is coupled to the activation of guanylate cyclase by an as yet undefined mechanism. Three subunit peptides may constitute a binding region of the receptor.

Kuno, T., Kamisaki, Y., Waldman, S. A., Gariepy, J., Schoonik, G., and *Murad, F.*

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Other support: National Insitutes of Health and the Veterans Administration.

From the Departments of Medicine, Pharmacology, and Microbiology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

COMPARISON OF BINDING AND CYCLIC GMP ACCUMULATION BY ATRIAL NATRIURETIC PEPTIDES IN ENDOTHELIAL CELLS

Rat ^{125}I-labeled atrial natriuretic factor (ANF (8–33)) was used to identify ANF receptors on cultured bovine aortic endothelial cells. Specific binding of ^{125}I-ANF at 37°C to confluent endothelial cells was saturable and of high affinity. Scatchard analysis of the equilibrium binding data indicated that endothelial cells contain a single class of binding sites with a K_d of 0.1 ± 0.01 nM. This particular clone of endothelial cells had 16000 ± 1300 receptors per cell. The order of potency for competing with ^{125}I-ANF binding was human atrial natriuretic peptide (hANP) = atrial natriuretic factor (ANF (8–33)) > atriopeptin II > atriopeptin III > atriopeptin. The weakest competitor, atriopeptin I, had a K_i of 0.45 nM, which was only 6-fold higher than the K_i for hANP and ANF (8–33). ANF (8–33) and hANP in the presence of 0.5 mM isobutylmethylxanthine produced a 15–20-fold increase in cyclic GMP content at 10 pM and a maximal 500-fold elevation of cyclic GMP at 10 nM. The concentrations required to elicit a half-maximal increase in cyclic GMP for hANP, ANF (8–33), atriopeptin I, atriopeptin II and atriopeptin III were 0.30, 0.35, > 500, 4.0 and 5.0 nM, respectively. Although atriopeptin I acted as a partial agonist, it was unable to antagonize the effect of ANF (8–33) on cyclic GMP formation. These findings suggest that endothelial cells have multiple and functionally distinct ANF-binding sites.

Leitman, D. C. and *Murad, F.*

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Other support: National Insitutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CO-PURIFICATION OF AN ATRIAL NATRIURETIC FACTOR RECEPTOR AND PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG

An atrial natriuretic factor (ANF) receptor from rat lung was solubilized with Lubrol-PX and purified by sequential chromatographic steps on GTP-agarose, DEAE-Sephacel, phenyl-agarose, and wheat germ agglutinin-agarose. The ANF receptor was enriched 19,000-fold. The purified receptor has a binding profile and properties that correspond to the affinity and specificity found in membranes and crude detergent extracts. Polyacrylamide gel electrophoresis of the purified preparation in the presence of sodium dodecyl sulfate and dithiothreitol showed the presence of one major protein band with a molecular mass of 120,000 daltons. When purified preparations were incubated with ^{125}I-ANF, then cross-linked with disuccinimidyl suberate, the 120,000-dalton protein was specifically radiolabeled. This high affinity binding site for ANF co-purified with particulate guanylate cyclase. Particulate guanylate cyclase was purified to a specific activity of 19 μmol cyclic GMP produced/min/mg of protein utilizing Mn-GTP as substrate. This represented a 15,000-fold purification compared to the initial lung membrane preparation with Lubrol-PX. Gel permeation high performance liquid chromatography and glycerol density gradient sedimentation studies of the purified preparation also resulted in co-migration of specific ANF binding and guanylate cyclase activities. The co-purification of these activities suggests that both ANF binding and guanylate cyclase activities reside in the same macromolecular complex. Presum-

ably ANF binding occurs at the external membrane surface and cyclic GMP synthesis at the internal membrane surface of this transmembrane glycoprotein.

Kuno, T., Andresen, J. W., Kamisaka, Y., Waldman, S. A., Chang, L. Y., Saheki, S., Leitman, D. C., Nakane, M., and *Murad, F.*

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Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

FORSKOLIN, PHOSPHODIESTERASE INHIBITORS, AND CYCLIC AMP ANALOGS INHIBIT PROLIFERATION OF CULTURED BOVINE AORTIC ENDOTHELIAL CELLS

The role of cyclic AMP on endothelial cell proliferation was investigated, since these cells can be exposed to high concentrations of physiological and pharmacological agents that alter cyclic AMP metabolism. Cloned bovine aortic endothelial cells were plated at 25,000 cells/35mm dish and grown for 5 days in the presence of phosphodiesterase (PDE) inhibitors, forskolin, or cyclic AMP analogs. The PDE inhibitors dipyridamole, ZK 62 711, isobutylmethylxanthine (IBMX) and theophylline inhibited cell growth in a concentration-dependent manner. Dipyridamole produced a 30% and a 50% inhibition at 5μM and 12.5μM, while higher concentrations were cytotoxic. At its therapeutic plasma concentration range (50–100μM) theophylline inhibited cell proliferation by 15–25%, while IBMX and the highly specific cyclic AMP phosphodiesterase inhibitor, ZK 62 711 inhibited growth by 60–80% and 40–50%, respectively. Forskolin (5μM) increased cyclic AMP levels and cyclic AMP-kinase activity ratios by 2.5-fold and 2-fold. In the absence of PDE inhibitors forskolin produced a 20% growth inhibition at 0.5μM and a 60% inhibition at 10μM. The forskolin dose-response curve was not altered by theophylline, but was shifted to the left by approximately 10-fold with dipyridamole and ZK 62 711 and 5-fold with IBMX. Forskolin (5μM), by itself produced a 1.8-fold increase in cyclic AMP. In the presence of 5μM theophylline, dipyridamole, IBMX, and ZK 62 711, cyclic AMP was increased by forskolin 2.0, 2.6, 3.5, and 6.6-fold, respectively. 8-Bromo cyclic AMP and dibutyryl cyclic AMP produced a 55% and 60% growth inhibition at 100μM. The cyclic GMP analogs were ineffective inhibitors of growth (15–30%). Our results demonstrate that cyclic AMP analogs and pharmacological agents that elevate intracellular cyclic AMP levels inhibit cell growth and suggest that cyclic AMP may be an important endogenous regulator of endothelial cell proliferation.

Leitman, D. C., Fiscus, R. R. and *Murad, F.*

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From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

DESENSITIZATION TO NITROGLYCERIN IN VASCULAR SMOOTH MUSCLE FROM RAT AND HUMAN

Cyanylate cyclase in high speed supernatant fractions obtained from rat thoracic aorta or human coronary arteries pretreated with nitroglycerin exhibited a marked desensitization to activation by nitroglycerin, nitroprusside, and nitric oxide. However, activation of soluble guanylate cyclase by arachidonic acid was unaffected by pretreatment of vessels with nitroglycerin. Furthermore, activation of soluble guanylate cyclase by protoporphyrin IX was increased 4-fold when vessels were pretreated with nitroglycerin. Soluble guanylate cyclase partially purified from nitroglycerin-pretreated rat thoracic aorta by immunoprecipitation with a specific monoclonal antibody exhibited persistent desensitization to nitrate-induced activation. These date suggest that nitroglycerin-induced desensitization of guanylate cyclase to activation by nitrovasodilators represents a stable alteration of the enzyme. In contrast, activation by protoporphyrin IX of guanylate cyclase immunoprecipitated from nitroglycerin-pretreated or control vessels was not significantly different. This suggests that the mechanism of protoporphyrin activation of guanylate cyclase is different than the mechanism with nitrovasodilators. Activation of particulate guanylate cyclase by Lubrol-PX, hemin, or atrial natriuretic factor was not significantly different with enzyme prepared from nitroglycerin-pretreated or control vessels from rat and human. Thus, nitroglycerin-induced desensitization or rat thoracic aorta or human coronary artery results in a relatively stable molecular alteration of soluble guanylate cyclase such that the enzyme is specifically less sensitive to activation by nitrovasodilators, whereas the effects of other activators of the enzyme are either unchanged or increased.

Waldman, S. A., Rapoport, R. M., Ginsburg, R, *Murad, F.*

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From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

IDENTIFICATION OF MULTIPLE BINDING SITES FOR ATRIAL NATRIURETIC FACTOR BY AFFINITY CROSS-LINKING IN CULTURED ENDOTHELIAL CELLS

In a previous study, we found that atriopeptin I was much weaker (EC_{50} > 500 nM) than atrial natriuretic factor (ANF-(8–33)) (EC_{50} = 0.3 nM) at increasing cyclic GMP in cultured endothelial cells. In this study, we used the cross-linking reagent disuccinimidyl suberate to investigate whether the differences in activity were due to the presence of multiple ANF receptors. When 98% of the ANF-binding sites on endothelial cells were occupied by tyrosine-atriopeptin I after cross-linking, there was no difference in the concentration-response curve to ANF-(8–33) with regard to cyclic GMP accumulation. In contrast, when 96% of the binding sites were occupied by cross-linked ANF-(8–33), a 60% decrease in the maximal cyclic GMP response was observed after the readdition of ANF-(8–33). These results suggest that ANF-(8–33) is binding to an additional site that atriopeptin I does not effectively bind. Affinity cross-linking of ^{125}I-ANF to intact endothelial cells resulted in the labeling of two sites of M_r ~66,000 and ~130,000. Approximately 94% of the ^{125}I-ANF binding sites had an M_r ~66,000. Labeling of this site was inhibited by both tyrosine-atriopeptin I (K_i = 0.9 nM) and

ANF-(8–33) $K_i = 0.9$ nM). Although 0.1 μM tyrosine-atriopeptin (AP I) inhibited labeling of the 66,000-dalton site to nearly the same degree as ANF-(8–33), it produced only a 4-fold increase in cyclic GMP compared to a 400-fold increase with ANF-(8–33). These results suggest that the 66,000-dalton site is not coupled to guanylate cyclase and cyclic GMP formation. Tyrosine-AP I ($K_i > 10$ nM) was much weaker at competing for the 130,000-dalton site than ANF-(8–33) ($K_i = 0.075$ nM). Because the EC_{50} for cyclic GMP stimulation for tyrosine-AP I (>100 nM) and ANF-(8–33) (0.4 nM) is closer to the K_i values for the 130,000-dalton protein, this site probably mediates the marked stimulation of cyclic GMP. Our results demonstrate that endothelial cells contain two binding sites for ANF-(8–33) and suggest that only the less abundant site ($M_r \sim 130,000$) is the receptor coupled to the activation of guanylate cyclase.

Leitman, D. C., Andresen, J. W., Kuno, T., and *Murad, F.*

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Other support: National Institutes of Health.

From the Department of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA.

EPR STUDIES SHOW THAT ALL LANTHANIDES DO NOT HAVE THE SAME ORDER OF BINDING TO CALMODULIN

Calmodulin, spin labeled at Tyr-99, has been titrated with the lanthanides La^{3+}, Nd^{3+}, Eu^{3+}, Tb^{3+}, Er^{3+} and Lu^{3+} as well as Ca^{2+} and Cd^{2+}. The titration was monitored by EPR and changes in mobility of the spin label, due to binding into the labeled site and protein conformational change, were observed. Comparison of these titration curves with theoretical binding curves for the various calmodulin-metal species, show that different lanthanides have different high affinity sites. Three basic categories were observed, with Lu^{3+} and Er^{3+} behaving like Ca^{2+}, Eu^{3+} and Tb^{3+} binding in the opposite order from Ca^{2+}, and La^{3+} and Nd^{3+} different from either Ca^{2+} or Tb^{3+}.

Buccigross, J. M. and *Nelson, D. J.*

Biochemical and Biophysical Research Communications **138**(3):1243-1249, 1986.

From the Department of Chemistry, Clark University, Worcester, MA.

A FLOW-DIALYSIS METHOD FOR OBTAINING RELATIVE MEASURES OF ASSOCIATION CONSTANTS IN CALMODULIN-METAL-ION SYSTEMS

A flow-dialysis apparatus suitable for the study of high-affinity metal-binding proteins has been utilized to study calmodulin–metal exchange as a measure of relative calmodulin–metal association constants. Calmodulin labelled with radioactive ^{153}Gd was dialysed against buffer containing various competing metal ions. The rate of label exchange was monitored by a γ-ray scintillation detector. Competing metals used were Ca^{2+} and Cd^{2+}, and the lanthanides Gd^{3+}, Eu^{3+}, La^{3+} and Lu^{3+}. All exchange processes were first-order, and two categories of metal were found: Ca^{2+} and Cd^{2+} in one, the lanthanides comprising the other. In addition calmodulin–metal complexes with radioactive ^{109}Cd and ^{45}Ca released the bound label without any competing metal being added to the buffer. The kinetics of this metal loss can be described by two

consecutive first-order processes, and the fraction of label associated with each rate can be determined. Studies of phosphodiesterase activation by calmodulin show Cd^{2+} and calmodulin to cause 80% of the maximum activation found when Ca^{2+} and calmodulin are used.

Buccigross, J. M., O'Donnell, C. L., and *Nelson, D. J.*

Biochemical Journal **235**:677-684, 1986.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

SOLVENT INTERACTIONS WITH *N,N*-DIALKYLNICOTINAMIDES AND THEIR EFFECTS ON ROTATIONAL BARRIERS

Carbon-13 nuclear magnetic resonance techniques were employed to examine the effects of solvent environment on rotational barriers in a series of molecules structurally-related to the analeptic, nikethamide: *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine. Total bandshape analysis was performed for the exchanging alkyl carbon resonances of these compounds as a function of temperature in four solvent systems: D_2O, CH_3OD, CH_3CH_2OD and $CDCl_3$. The rate constants for rotation about the amide bond obtained in this way were used to calculate free energy ($\Delta G\ddagger$), enthalpy ($\Delta H\ddagger$) and entropy ($\Delta S\ddagger$) of activation parameters for this process. Our results indicate that rotational barriers are less affected by the nature of the alkyl chain attached to the amide nitrogen than by the size and polarity of the solvent molecules. Interpretation of the thermodynamic parameters in light of both nikethamide analogue structure and solvent type has further clarified the manner in which hydrogen bonding interactions between solvent molecules and the carbonyl oxygen of these analogues stabilize transition state conformers.

Bean, J. W., *Nelson, D. J.*, and Wright, G. E.

Biochemical Pharmacology **35**(6):1011-1017, 1986.

Other support: National Science Foundation.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

$^{153}Eu^{3+}$ AS A PROBE OF METAL ION AND CATIONIC DRUG BINDING SITES ON NATIVE AND HEAT-DENATURED DNA

A flow-dialysis apparatus suitable for the study of high affinity metal ion binding sites in macro-molecules has been utilized to study $^{153}Eu^{3+}$ exchange processes, as a function of pH, in both "native" and "heat-denatured" DNA. "Free exchange" of $^{153}Eu^{3+}$ was found to occur at a significantly faster rate at pH = 7.0 than at pH = 6.0 for both forms of DNA; while non-radioactive $^{153}Eu^{3+}$-induced "displacement" of bound $^{153}Eu^{3+}$ occurred at a significantly faster rate at pH = 6.0 than at pH = 7.0 for both species of DNA. These results are consistent with a greater "entropic" driving force for metal ion:DNA complexation at the lower pH value. The effect of ethidium bromide on $^{153}Eu^{3+}$ exchange was also examined as a function of pH. The intercalating agent was found to accelerate $^{153}Eu^{3+}$ displacement at pH = 6.0 and decelerate dis-

placement at pH = 7.0. All three sets of experiments, (*i.e.*, free-exchange of bound $^{152}Eu^{3+}$, $^{153}Eu^{3+}$-induced displacement of bound $^{152}Eu^{3+}$ and ethidium ion-induced displacement of bound $^{152}Eu^{3+}$) indicate that the $^{152}Eu^{3+}$ ion can serve as a useful probe of metal ion and drug binding sites in nucleic acid polymers and constitutes a particularly sensitive probe at pH = 6.0.

Rosenthal, L. S., and *Nelson, D. J.*

Inorganica Chimica Acta **125**: 89-95, 1986.

Other support: National Science Foundation.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

FURTHER CHARACTERIZATION OF THE PLATINUM-REACTIVE COMPONENT OF THE α_2-MACROGLOBULIN-RECEPTOR RECOGNITION SITE

α_2-Macroglobulin (α_2M)–methylamine that had been allowed to react with *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) bound with greatly reduced affinity to specific α_2M receptors, as determined by macrophage binding studies *in vivo* and plasma-clearance experiments *in vivo*. Subsequent reaction with diethyl dithiocarbamate completely restored receptor recognition function. The optimal effect was obtained when the diethyl dithiocarbamate concentration was twice the total platinum concentration. α_2M–methylamine that was allowed to react with H_2O_2 competed less effectively for specific cell-surface binding sites, as demonstrated by studies both *in vivo* and *in vitro*. The apparent dissociation constant was increased nearly 7-fold by a 15 min exposure to H_2O_2. α_2M–methylamine was affected significantly less by the H_2O_2 exposure after pretreatment with *cis*-DDP. Amino acid analysis indicated that H_2O_2 treatment of α_2M modified 19 of the 25 methionine residues per α_2M subunit. Pretreatment with *cis*-DDP protected two to four of these methionine residues. The only other residue altered by H_2O_2 treatment of α_2M was histidine. A net decrease of two histidine residues per subunit was observed, but *cis*-DDP pretreatment did not alter this result. In order to rule out the slight possibility that histidine modification might account for the observed H_2O_2-induced loss in receptor recognition, diethyl pyrocarbonate was employed as a histidine-modifying reagent. This treatment modified 53 histidine residues in both native and fast-form α_2M. Fast-form α_2M was still recognized by the α_2M receptor, as determined by studies both *in vivo* and *in vitro*; however, a fraction of the modified protein now cleared via the acyl-low-density-lipoprotein receptor as well. Reaction of diethyl pyrocarbonate-treated α_2M with hydroxylamine reversed derivatization of 43 of the 53 histidine residues. Moreover, this treatment also resulted in an α_2M fast-form preparation that was recognized only by the α_2M receptor. It is concluded that *cis*-DDP and H_2O_2 modify a critical methionine residue in the primary sequence of the α_2M-receptor recognition site.

Pizzo, S. V. et al.

Biochemical Journal **238**:217-225, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology and Biochemistry, Duke University Medical Center, Durham, NC.

THE MECHANISMS OF THE INACTIVATION OF HUMAN ALPHA-1-PROTEINASE INHIBITOR BY GAS-PHASE CIGARETTE SMOKE

Cigarette smoke, either directly or indirectly, causes alpha-1-proteinase inhibitor (αIPI) to lose elastase inhibitory capacity (EIC), leaving lung connective tissues susceptible to proteolytic degradation. This paper discusses possible mechanisms for inactivation by cigarette smoke (CS) and by a model system [NO, isoprene, and air] that duplicates much of CS free radical chemistry. Inactivation of αIPI by either CS or the model is biphasic, a fast inactivation is followed by a slower one. With pre-prepared extracts, only the slow inactivation is observed. Apparently short-lived species in the smoke itself and the model system cause the fast inactivation; they may be peroxynitrates, which form in smoke from nitrogen dioxide and peroxyl radicals. The slower inactivation appears to involve hydrogen peroxide and/or organic hydroperoxides or species produced by them. Incubation of αIPI with linoleic acid produces a slow loss of EIC, prevented by the presence of vitamin E, which supports the hypothesis of a route involving lipid hydroperoxides. Protection of αIPI by various types of compounds shows that unprotonated amines and amino acids protect, but the protonated or acylated compounds do not. Ascorbate and glutathione provide the strongest protection.

Pryor, W. A., et al.

Advances in Free Radical Biology & Medicine 2:161-188, 1986.

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Biodynamics Institute, Louisiana State University, Baton Rouge.

USE OF ENDOTHELIAL CELLS IN CULTURE FOR STUDIES OF THE MICROCIRCULATION

Physiological studies have indicated that endothelial cells, especially those of the lungs, play an active role in the processing of blood-borne vasoactive substances, including adenine nucleotides, biogenic amines and polypeptide hormones. Studies using cultures of pure lines of pulmonary endothelial cells confirm the hypothesis that circulating ATP, ADP, AMP are metabolized by enzymes on the luminal surface of endothelial cells. Cytochemical localization at the electron microscope level shows that the enzymes responsible are located in those endothelial caveolae that directly face the vascular lumen. Likewise, pure cultures of endothelial cells metabolize bradykinin and angiotensin I to yield the same products as do intact lungs. Immunocytochemical localization at the electron microscope level indicates that angiotensin converting enzyme (EC 3.4.15.1), the enzyme responsible for both degradation of bradykinin and conversion of angiotensin I to angiotensin II, is located on both caveolae and on undifferentiated portions of the endothelial plasma membrane. The enzyme is located with its active site directly accessible to plasma substrates. Endothelial cells in culture provide a means for studying endothelial properties without the complication of enzymic contributions from other cell types. For example, endothelial cells possess, and probably synthesize, surface enzymes such as ATPase, ADPase, 5'-nucleotidase,

angiotensin converting enzyme, carboxypeptidase N, and carbonic anyhydrase; enzyme inhibitors such as α_2-macroglobulin; receptors for bradykinin and other hormones; intracellular enzymes for synthesis of prostaglandins and for the synthesis of extracellular products such as components of the glycocalyx and basement membranes. Endothelial cells of the lungs play an active role in determining the quality of systemic arterial blood. Endothelial vesicles that open directly to the vascular space provide an ultrastructural specialization that is ideally suited for favoring interaction between solutes and colloids of plasma with endothelial cell-bound enzymes.

Ryan, U. S.

Progress in Applied Microcirculation **9**:150-164. 1985.

Other support: National Insitutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

HPLC ANALYSIS OF AMINO ACIDS WITH ION EXCHANGE CHROMATOGRAPHY AND O-PHTHALALDEHYDE POST-COLUMN DERIVATIZATION: APPLICATIONS TO THE ASSAY OF ENDOGENOUS FREE AMINO ACIDS AND THEIR TRANSPORT IN HUMAN PLACENTAL VILLUS

A method using high performance liquid chromatography (HPLC) for the analysis of primary amino acids in human placenta is described. This method involves separation of primary amino acids by high performance ion-exchange chromatography followed by post column derivatization using O-phthalaldehyde (OPA) and 2-mercaptoethanol and fluorescence (excitation 340 nm and emission 410 nm) detection of derivatives. Waters 840 HPLC Amino Acid System was used for this purpose. For analysis, villus tissue was extracted with acetonitrile, and the recovered amino acids were reconstituted in a sodium diluent (pH 2.2). The complete profile of the primary amino acids in the sample could be constructed in about 90 minutes. Up to 44 samples can be analyzed without special attention. Using this method, essential amino acids (threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine) and nonessential amino acids (aspartic acid, serine, glutamic acid, glycine, alanine, arginine) were detected and quantified in human placental villus in pmol quantities. Plots of peak heights (or areas) were linear for several amino acids. The same method was also used for (a) the assay of free primary amino acids in umbilical bloods, (b) the efflux of amino acids from isolated human placental villus, and (c) to study the uptake of α-aminoisobutyric acid (AIB), a non-metabolizable amino acid, by the isolated placental villus.

Sastry, B. V. R., et al.

Journal of Liquid Chromatography **9**(8):1689-1710, 1986.

Other support: National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS ON CALCIUM AND CENTRILOBULAR NECROSIS IN THE LIVER OF RATS TREATED WITH HEPATOTOXIC AGENTS

Carbon tetrachloride, chloroform, dimethylnitrosamine, thioacetamide or acetaminophen was each administered to rats in a single hepatotoxic dose. Nifedipine, verapamil or chlorpromazine was administered in association with the hepatotoxic agents to determine if calcium channel blocking agents would prevent an increase in liver cell calcium associated with hepatotoxicity and to determine if these agents would protect against the development of centrilobular necrosis. Following a latent period different for each toxic agent, a 4- to 18-fold increase in liver cell calcium content had occurred by 24 hr. The calcium increase and the centrilobular necrosis (mean histologic score) were correlated. A relatively high calcium to necrosis ratio was obtained with dimethylnitrosamine, thioacetamide and acetaminophen. A lesser calcium to necrosis ratio was obtained with chloroform and carbon tetrachloride, the two toxic agents that destroyed the intracellular calcium sequestration activity of the liver endoplasmic reticulum. Nifedipine or chlorpromazine, administered prior to and 7 hr after the toxic agent, completely prevented the centrilobular necrosis caused by thioacetamide, carbon tetrachloride and acetaminophen; almost completely prevented necrosis with dimethylnitrosamine; and provided partial protection against chloroform toxicity. Two doses of verapamil provided partial protection against necrosis when carbon tetrachloride was the toxic agent and provided almost complete protection with dimethylnitrosamine. A reduction in liver cell calcium was associated with the protective action of the three calcium channel blocking agents. These findings are compared with earlier studies of the protective effects of calcium channel blocking agents in cardiac ischemia.

Landon, E. J., Naukam, R. J., and *Sastry, B. V. R.*

Biochemical Pharmacology **35**(4):697-705, 1986.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

ALTERED KINETIC PROPERTIES OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE FOLLOWING DIETARY MANIPULATIONS

The microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the rate-limiting step in the cholesterogenic pathway and was proposed to be composed *in situ* of 2 noncovalently linked subunits. In the present report, the activities and kinetic properties of HMG-CoA reductase in microsomes isolated from livers of rats fed on diets supplemented with either ground Amberlite XAD-2 ("X"), cholestyramine/mevinolin ("CM"), or unsupplemented normal rat chow ("N"), were compared.

The specific activities of HMG-CoA reductase in X and CM microsomes were, respectively, 5- and 83-fold higher than that of N microsomes. In NADPH-dependent kinetics of HMG-CoA reductase activated with 4.5 mM GSH, the concentration of NADPH required for half-maximal velocity ($S_{0.5}$) was 209 ± 23, 76 ± 23, and 40 ± 4 μM for the N, X, and CM microsomes, respectively. While reductase from X microsomes

displays cooperative kinetics toward NADPH (Hill coefficient (n_H) = 1.97 ± 0.07), the enzyme from CM microsomes does not (n_H) = 1.04 ± 0.07). Similarly to HMG-CoA reductase from CM microsomes, the freeze-thaw solubilized enzyme ("SOL") displays no cooperativity toward NADPH and its K_m for this substrate is 34 μM.

At 4.5 mM GSH, HMG-CoA reductase from X, CM, and SOL preparations has a similar K_m value for [DL]-HMG-CoA, ranging between 13–16 μM, while reductase from N microsomes had a higher K_m value (42 μM) for this substrate. No cooperativity towards HMG-CoA was observed in any of the tested enzyme preparations.

Immunoblotting analyses of the different preparations demonstrated that the observed altered kinetics of HMG-CoA reductase in the microsomes are not due to preferential proteolytic cleavage of the native 97–100 kDa subunit of the enzyme to the noncooperative 50–55 kDa species. Moreover, it was found that the ratio enzymatic activity/immunoreactivity of the reductase increased in the order N<X<CM≅SOL, indicating that the activity per reductase molecule increases with the induction of the enzyme. These results are compatible with a model suggesting that dietary induction of hepatic HMG-CoA reductase may change the state of functional aggregation of its subunits.

Roitelman, J. and *Shechter, I.*

The Journal of Biological Chemistry **261**(11):5061-5066, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

STRUCTURE AND MOBILITY OF ACTIN FILAMENTS AS MEASURED BY QUASIELASTIC LIGHT SCATTERING, VISCOMETRY AND ELECTRON MICROSCOPY

Actin filaments of different lengths were prepared by polymerizing actin in the presence of various concentrations of gelsolin, a protein which accelerates actin polymerization by stabilizing nuclei from which filaments grow and which binds to their fast growing ends. The lengths of the actin filaments following polymerization were measured by electron microscopy and showed that the number-average filament length agreed with the predicted length if each gelsolin molecule acted as a seed for the growth of an actin filament. The distribution of lengths was independent of the actin: gelsolin ratio and was similar to that of actin filaments polymerized in the absence of gelsolin ($L_w/L_n = 1.8$). The mobility of these filaments in solution was studied by quasielastic light scattering and by viscometry. The translational diffusion constant determined by quasielastic light scattering was in agreement with the infinite dilution values calculated from the dimensions and the distributions of lengths determined by electron microscopy for relatively short filament lengths. Under conditions where overlap of the rotational domains of the filaments would be expected to occur, the measured diffusion rates deviated from their predicted dilute solution values and the solution viscosity increased abruptly. The dependence of the diffusion constant and the solution viscosity on the length of the actin filaments can be explained in terms of a theory that describes the restraints on diffusion of independent rigid rods in semi-dilute solution. The results suggest that the rheology of actin filaments can be accounted for by steric restraints. The length of cytoplasmic actin filaments in some cell types is such that these steric constraints are significant and could produce large changes in physical properties with small changes in filament length.

Janmey, P. A., Peetermans, J., Zaner, K. S. and *Stossel, T. P.*

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Other support: U. S. Public Health Service and the Richard Saltonstall Endowment.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

THE ARCHITECTURE OF ACTIN FILAMENTS AND THE ULTRASTRUCTURAL LOCATION OF ACTIN-BINDING PROTEIN IN THE PERIPHERY OF LUNG MACROPHAGES

A highly branched filament network is the principal structure in the periphery of detergent-extract cytoskeletons of macrophages that have been spread on a surface and either freeze or critical point dried, and then rotary shadowed with platinum-carbon. This array of filaments completely fills lamellae extended from the cell and bifurcates to form 0.2-0.5 μm thick layers on the top and bottom of the cell body. Reaction of the macrophage cytoskeletons with anti-actin IgG and with anti-IgG bound to colloidal gold produces dense staining of these filaments, and incubation with myosin subfragment I uniformly decorates these filaments, identifying them as actin. 45% of the total cellular actin and ~70% of actin-binding protein remain in the detergent-insoluble cell residue. The soluble actin is not filamentous as determined by sedimentation analysis, the DNAase I inhibition assay, and electron microscopy, indicating that the cytoskeleton is not fragmented by detergent extraction. The spacing between the ramifications of the actin network is 94 ± 47 nm and 118 ± 72 nm in cytoskeletons prepared for electron microscopy by freeze drying and critical point drying, respectively. Free filament ends are rare, except for a few which project upward from the body of the network or which extend down to the substrate. Filaments of the network intersect predominantly at right angles to form either T-shaped and X-shaped overlaps having striking perpendicularity or else Y-shaped intersections composed of filaments intersecting at 120-130° angles. The actin filament concentration in the lamellae is high, with an average value of 12.5 mg/ml. The concentration was much more uniform in freeze-dried preparations than in critical point-dried specimens, indicating that there is less collapse associated with the freezing technique.

The orthogonal actin network of the macrophage cortical cytoplasm resembles actin gels made with actin-binding protein. Reaction of cell cytoskeletons and of an actin gel made with actin-binding protein with anti-actin-binding protein IgG and anti-IgG-coated gold beads resulted in the deposition of clusters of gold at points where filaments intersect and at the ends of filaments that may have been in contact with the membrane before its removal with detergent. In the actin gel made with actin-binding protein, 75% of actin-fiber intersections labeled, and the filament spacing between intersections was consistent with that predicted on theoretical grounds if each added actin-binding protein molecule cross-links two filaments to form an intersection in the gel. On the other hand, only 38% of all filament intersections in the macrophage cortical cytoskeletons labeled, and the filament spacing between junctions was much smaller than that predicted from the molar ratio of actin binding protein to actin in the cytoskeletons. Despite the differences between actin gels made with actin-binding protein and macrophage cytoskeletons, which presumably arise from the greater com-

plexity of the cell compared with the purified protein assembly, the networks were qualitatively similar.

Hartwig, J. H. and Shevlin, P. (*Stossel, T.*)

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Other support: National Institutes of Health, National Science Foundation and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

PROBING THE MECHANISM OF INCORPORATION OF FLUORESCENTLY LABELED ACTIN INTO STRESS FIBERS

The mechanism of actin incorporation into and association with stress fibers of 3T3 and WI38 fibroblasts was examined by fluorescent analog cytochemistry, fluorescence recovery after photobleaching (FRAP), image analysis, and immunoelectron microscopy. Microinjected, fluorescein-labeled actin (AF-actin) became associated with stress fibers as early as 5 min post-injection. There was no detectable cellular polarity in the association of AF-actin with pre-existing stress fibers relative to perinuclear or peripheral regions. The rate of incorporation was quantified by image analysis of images generated with a two-dimensional photon counting microchannel plate camera. After equilibration of up to 2 h post-injection, FRAP demonstrated that actin subunits exchanged rapidly between filaments in stress fibers and the surrounding cytoplasm. When co-injected with rhodamine-labeled bovine serum albumin as a control, only actin was detected in the phase-dense stress fibers. The control protein was excluded from fibers and any linear fluorescence of the control was demonstrated as a pathlength artifact. The incorporation of AF-actin into stress fibers was studied by immunoelectron microscopy using anti-fluorescein as the primary antibody and goat anti-rabbit IgG coupled to peroxidase as the secondary antibody. At 5 min post-injection, reaction product was localized periodically in some fibers with a periodicity of ~0.75 μm. In large diameter fibers at 5 min post-injection, the analog was seen first on the surface of fibers, with individual filaments resolvable within the core. In the same cell, thinner diameter fibers were labeled uniformly throughout the diameter. By 20 min post-injection, most fibers were uniformly labeled. We conclude that the rate of actin subunit exchange *in vivo* is extremely rapid with molecular incorporation into actin filaments of stress fibers occurring as early as a few minutes post-injection. Exchange appears to first occur in filaments along the surface of stress fibers and then into more central regions in a periodic manner. We suggest that the periodic localization of actin at very early time points is due to a local microheterogeneity in which microdomains of fast vs. slower incorporation result from the periodic localization of actin-binding protein, such as α-actinin, along the length of the fiber.

Amato, P. A. and *Taylor, D. L.*

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Other support: National Institutes of Health.

From the Department of Biological Sciences and Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

IMAGING AT LOW LIGHT LEVEL IN FLUORESCENCE MICROSCOPY

Quantitative fluroescence microscopy is a powerful tool for studying the spatial and temporal dynamics of macromolecules, molecules and ions in living cells for at least five important reasons: (1) Sensitivity—with existing technology, as few as ca. 50 fluorescent molecules can be detected as a cluster on surfaces or inside cells. (2) Specificity—single classes of molecules can be studied even in a mixture of many other molecules by selecting the proper fluorescent probes and filter combinations. (3) Spectroscopy—fluorescence spectroscopic measurements such as fluorescence anisotropy and resonance energy transfer can yield molecular information about the molecules or the immediate environment. (4) Spatial resolution—the use of the fluorescence microscope permits the analysis of fluorescence parameters at the limit of resolution of the light microscope. Therefore, two- and three-dimensional maps of molecular distributions, as well as molecular activity, are possible in living cells. (5) Temporal resolution—fluorescence techniques are also valuable since they can detect and quantify those events which occur with a rate on the order of about 10^{9} sec^{-1} or slower.

The authors discuss some of the parameters that must be considered when a low-light-level video microscope system is used for quantitative imaging of fluorescent molecules. The parameters discussed are fundamental both for characterizing the limits of capability of the system as well as for providing the necessary data for partially rectifying some of these limitations by using image processing techniques.

Bright, G. R., and *Taylor, D. L.*

In: *Applications of Fluorescence in the Biomedical Sciences*, Alan R. Liss, Inc., 1986, Chap. 12, pp. 257-288.

Other support: National Institutes of Health and the Leukemia Society.

From the Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

FC-RECEPTOR-MEDIATED PHAGOCYTOSIS OCCURS IN MACROPHAGES WITHOUT AN INCREASE IN AVERAGE $[Ca^{++}]_i$

The calcium ion has been implicated as a cytosolic signal or regulator in phagocytosis. Using the Ca^{++}-sensitive photoprotein aequorin we have measured intracellular free Ca^{++} ion concentration ($[Ca^{++}]_i$) in thioglycolate-elicited mouse peritoneal macrophages during phagocytosis and IgG-induced spreading. Macrophages plated on glass were loaded with aequorin and $[Ca^{++}]_i$ was then measured from cell populations, both as previously described (McNeil, P. L., and D. L. Taylor, 1985, *Cell Calcium*, 6:83–92). Aequorin indicated a resting $[Ca^{++}]_i$ in adherent macrophages of 84 nM and was responsive to changes in $[Ca^{++}]_i$ induced by the addition of Mg-ATP (0.1 mM) or serum to medium. However, during the 15 min required for phagocytosis of seven or eight IgG-coated erythrocytes per macrophage loaded with aequorin, we measured no change in $[Ca^{++}]_i$. Similarly, the ligation of Fc-receptors that occurs when macrophages spread on immune complex-coated coverslips did not change macrophage $[Ca^{++}]_i$. In contrast, a rise in $[Ca^{++}]_i$ of macrophages was measured during phagocytosis occurring in a serum-free saline of pH 7.85, and as a consequence of incubation

with quin2/AM. We estimate that had a change in $[Ca^{++}]$ occurred during phagocytosis, aequorin would have detected a rise from 0.1 to 1.0 μM taking place in as little as 2% of the macrophage's cytoplasmic volume. We therefore suggest that either Ca^{++} is not involved as a cytoplasmic signal for phagocytosis or that increases in $[Ca^{++}]$ during phagocytosis are confined to such small regions of cytoplasm as to be below the limits of detection by our cellular averaging method. Our data emphasizes, moreover, the need for well-defined, nonperturbing conditions in such measurements of $[Ca^{++}]$.

McNeil, P. L., Swanson, J. A., Wright, S. D., and *Taylor, D. L.*

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Other support: U. S. Public Health Service and the American Cancer Society.

From the Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

A TRANSIENT RISE IN CYTOSOLIC CALCIUM FOLLOWS STIMULATION OF QUIESCENT CELLS WITH GROWTH FACTORS AND IS INHIBITABLE WITH PHORBOL MYRISTATE ACETATE

We have used aequorin as an indicator for the intracellular free calcium ion concentration ($[Ca^{++}]_i$) of Swiss 3T3 fibroblasts. Estimated $[Ca^{++}]_i$ of serum-deprived, subconfluent fibroblasts was 89 (±20) nM, almost twofold higher than that of subconfluent cells growing in serum, whose $[Ca^{++}]_i$ was 50 (±19) nM. Serum, partially purified platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) stimulated DNA synthesis by the serum-deprived cells, whereas epidermal growth factor (EGF) did not. Serum immediately and transiently elevated the $[Ca^{++}]_i$ of serum-deprived cells, which reached a maximal value of 5.3 μM at 18 s poststimulation but returned to near prestimulatory levels within 3 min. Moreover, no further changes in $[Ca^{++}]_i$ were observed during 12 subsequent h of continuous recording. PDGF produced a peak rise in $[Ca^{++}]_i$ to ~1.4 μM at 115 s after stimulation, and FGF to ~1.2 μM at 135 s after stimulation. EGF caused no change in $[Ca^{++}]_i$. The primary source of calcium for these transients was intracellular, since the magnitude of the serum-induced rise in $[Ca^{++}]_i$ was reduced by only 30% in the absence of exogenous calcium. Phorbol 12-myristate 13-acetate (PMA) had no effect on resting $[Ca^{++}]_i$. When, however, quiescent cells were treated for 30 min with 100 nM PMA, serum-induced rises in $[Ca^{++}]_i$ were reduced by sevenfold. PMA did not inhibit growth factor-induced DNA synthesis and was by itself partially mitogenic. We suggest that if calcium is involved as a cytoplasmic signal for mitogenic activation of quiescent fibroblasts, its action is early, transient, and can be partially substituted for by PMA. Activated protein kinase C may regulate growth factor-induced increases in $[Ca^{++}]_i$.

McNeil, P. L., McKenna, M. P. and *Taylor, D. L.*

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Other support: National Institutes of Health.

Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon, Pittsburgh.

HUMAN LEUKOCYTE CATHEPSIN G. SUBSITE MAPPING WITH 4-NITROANILIDES, CHEMICAL MODIFICATION, AND EFFECT OF POSSIBLE COFACTORS

The extended substrate binding site of cathepsin G from human leukocytes has been mapped by using a series of peptide 4-nitroanilide substrates. The enzyme has a significant preference for substrates with a P_1 Phe over those with the other aromatic amino acids Tyr and Trp. The S_2 subsite was mapped with the substrates Suc-Phe-AA-Phe-NA where AA was 13 of the 20 amino acid residues commonly found in proteins. The best residues were Pro and Met. The S_3 subsite was mapped with the sequence Suc-AA-Pro-Phe-NA by using 14 different amino acid residues for AA. The two best residues were the isosteric Val and Thr. No significant improvement in reactivity was obtained by extending the substrate to include seven different P_4 residues. The kinetic parameters for cathepsin G are significantly slower than those for many other serine proteases. Changes in the reaction conditions and addition of possible cofactors or ligands were in general found to have little effect on the enzymatic activity, while chemical modifications and proteolysis destroyed the activity of cathepsin G. Cathepsin G hydrolyzed peptides containing model desmosine residues and prefers the hydrophobic picolinoyllysine derivative over lysine by substantial margins at both the S_1 and S_2 subsites but will not tolerate it at S_3. Substrates with sequences related to the cathepsin G cleavage site in angiotensin I and angiotensinogen, and the reactive site of α_1-antichymotrypsin, were hydrolyzed effectively by enzyme, but with unexceptional rates. Our results indicate that the natural substrate(s) and function(s) of cathepsin G still remain to be discovered.

Tanaka, T., Minematsu, Y., Reilly, C. F., and *Travis, J.*

Biochemistry **24**:2040-2047, 1985.

From the Department of Biochemistry, University of Georgia, Athens.

INHIBITION OF HUMAN LEUKOCYTE ELASTASE, CATHEPSIN G, CHYMOTRYPSIN A_α, AND PORCINE PANCREATIC ELASTASE WITH SUBSTITUTED ISOBENZOFURANONES AND BENZOPYRADIONES

Several 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones, 3-(1-haloalkylidene)-1(3*H*)-isobenzofuranones, and 3-bromomethyl-1*H*-2-benzopyran-1-ones containing masked halo ketone functional groups were synthesized and tested as inhibitors of several serine proteases including human leukocyte (HL) elastase and cathepsin G. While many of the 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones were quite potent inhibitors of the enzymes tested, the alkylideneisobenzofuranones and benzopyran-1-ones inhibited poorly or not at all. The 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones decomposed rapidly upon addition to buffer to give the corresponding 3-alkyl-1*H*-2-benzopyran-1,4(3*H*)-diones. The pure benzopyran-1,4-diones were extremely potent inhibitors of HL elastase and chymotrypsin A_α but did not inactivate porcine pancreatic elastase or cathepsin G. Enzymes inhibited by the isobenzofuranones and benzopyran-1,4-diones regained activity slowly upon standing or after dialysis ($t_{1/2}$ = 5–16 h) and more rapidly in the presence of 0.5 M hydroxylamine, which indicated the presence of labile acyl moieties in the inhibited enzyme. These results are consistent with a scheme in which the active site serine of the protease reacts with the lactone carbonyl of these inhibitors to give a stable acyl enzyme and alkylation

of another active site residue by the unmasked halo ketone functional group does not occur.

Hemmi, K., Harper, J. W., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:1841-1848, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

MAMMALIAN CHYMOTRYPSIN-LIKE ENZYMES. COMPARATIVE REACTIVITIES OF RAT MAST CELL PROTEASES, HUMAN AND DOG SKIN CHYMASES, AND HUMAN CATHEPSIN G WITH PEPTIDE 4-NITROANILIDE SUBSTRATES AND WITH PEPTIDE CHLOROMETHYL KETONE AND SULFONYL FLUORIDE INHIBITORS

The extended substrate binding sites of several chymotrypsin-like serine proteases, including rat mast cell proteases I and II (RMCP I and II, respectively) and human and dog skin chymases, have been investigated by using peptide 4-nitroanilide substrates. In general, these enzymes preferred a P_1 Phe residue and hydrophobic amino acid residues in P_2 and P_3. A P_2 Pro residue was also found to be quite acceptable. The S_4 subsites of these enzymes are less restrictive than the other subsites investigated. The substrate specificity of these enzymes was also investigated by using substrates which contain model desmosine residues and peptides with amino acid sequences of the physiologically important substrates angiotensin I and angiotensinogen and α_1-antichymotrypsin, the major plasma inhibitor for chymotrypsin-like enzymes. These substrates were less reactive than the most reactive tripeptide reported here, Suc-Val-Pro-Phe-NA. The thiobenzyl ester Suc-Val-Pro-Phe-SBzl was found to be an extremely reactive substrate for the enzymes tested and was 6–171-fold more reactive than the 4-nitroanilide substrate. The four chymotrypsin-like enzymes were inhibited by chymostatin and N-substituted saccharin derivatives which had K_i values in the micromolar range. In addition, several potent peptide chloromethyl ketone and substituted benzenesulfonyl fluoride irreversible inhibitors for these enzymes were discovered. The most potent sulfonyl fluoride inhibitor for RMCP I, RMCP II, and human skin chymase, 2-(Z-NHCH$_2$CONH)C$_6$H$_4$SO$_2$F, had $k_{obs}/[I]$ values of 2500, 270, and 1800 $M^{-1}s^{-1}$, respectively. The substrates and inhibitors reported here should be extremely useful in elucidating the physiological roles of these proteases.

Powers, J. C., Tanaka, T., Harper, J. W., and Minematsu, Y. (*Travis, J.*)

Biochemistry **24**:2048-2058, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

REACTION OF SERINE PROTEASES WITH SUBSTITUTED ISOCOUMARINS: DISCOVERY OF 3,4-DICHLOROISOCOUMARIN, A NEW GENERAL MECHANISM BASED SERINE PROTEASE INHIBITOR

The mechanism-based inactivations of a number of serine proteases, including human leukocyte (HL) elastase, cathepsin G, rat mast cell proteases I and II, several

human and bovine blood coagulation proteases, and human factor D by substituted isocoumarins and phthalides which contain masked acyl chloride or anhydride moieties, are reported. 3,4-Dichloroisocoumarin, the most potent inhibitor investigated here, inactivated all the serine proteases tested but did not inhibit papain, leucine aminopeptidase, or β-lactamase. 3,4-Dichloroisocoumarin was fairly selective toward HL elastase ($K_{inact}/[I]$ = 8920 M^{-1} s^{-1}); the inhibited enzyme was quite stable to reactivation (K_{react} = 2 × 10^{-5} s^{-1}), while enzymes inhibited by 3-acetoxyisocoumarin and 3,3-dichlorophthalide regained full activity upon standing. The rate of inactivation was decreased dramatically in the presence of reversible inhibitors or substrates, and ultraviolet spectral measurements indicate that the isocoumarin ring structure is lost upon inactivation. Chymotrypsin A_γ is totally inactivated by 1.2 equiv of 3-chloroisocoumarin or 3,4-dichloroisocoumarin, and approximately 1 equiv of protons is released upon inactivation. These results indicate that these compounds react with serine proteases to release a reactive acyl chloride moiety which can acylate another active site residue. These are the first mechanism-based inhibitors reported for many of the enzymes tested, and 3,4-dichloroisocoumarin should find wide applicability as a general serine protease inhibitor.

Harper, J. W., Hemmi, K., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:1831-1841, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

REACTION OF SERINE PROTEASES WITH SUBSTITUTED 3-ALKOXY-4-CHLOROISOCOUMARINS AND 3-ALKOXY-7-AMINO-4-CHLOROISOCOUMARINS: NEW REACTIVE MECHANISM-BASED INHIBITORS

The time-dependent inactivation of several serine proteases including human leukocyte elastase, cathepsin G, rat mast cell proteases I and II, and human skin chymase by a number of 3-alkoxy-4-chloroisocoumarins, 3-alkoxy-4-chloro-7-nitroisocoumarins, and 3-alkoxy-7-amino-4-chloroisocoumarins at pH 7.5 and the inactivation of several trypsin-like enzymes including human thrombin and factor XIIa by 7-amino-4-chloro-3-ethoxyisocoumarin and 4-chloro-3-ethoxyisocoumarin are reported. The 3-alkoxy substituent of the isocoumarin is likely interacting with the S_1 subsite of the enzyme since the most reactive inhibitor for a particular enzyme had a 3-substituent complementary to the enzyme's primary substrate specificity site (S_1). Inactivation of several enzymes including human leukocyte elastase by the 3-alkoxy-7-amino-4-chloroisocoumarins is irreversible, and less than 3% activity is regained upon extensive dialysis of the inactivated enzyme. Addition of hydroxylamine to enzymes inactivated by the 3-alkoxy-7-amino-4-chloroisocoumarins results in a slow ($t_{1/2}$ > 6.7 h) and incomplete (32–57%) regain in enzymatic activity at pH 7.5. Inactivation by the 3-alkoxy-4-chloroisocoumarins and 3-alkoxy-4-chloro-7-nitroisocoumarins on the other hand is transient, and full enzyme activity is regained rapidly either upon standing, after dialysis, or upon the addition of buffered hydroxylamine. The rate of inactivation by the substituted isocoumarins is decreased when substrates or reversible inhibitors are present in the incubation mixture, which indicates active site involvement. The inactivation rates are dependent upon the pH of the reaction mixture, the isocoumarin ring system is opened concurrently with inactivation, and the reaction of

3-alkoxy-7-amino-4-chloroisocoumarins with porcine pancreatic elastase is shown to be stoichiometric. The results are consistent with a scheme where 3-alkoxy-7-amino-4-chloroisocoumarins react with the active site serine of a serine protease to give an acyl enzyme in which a reactive quinone imine methide can be released. Irreversible inactivation could then occur upon alkylation of an active site nucleophile (probably histidine-57) by the acyl quinone imine methide. The finding that hydroxylamine slowly catalyzes partial reactivation indicates that several inactivated enzyme species may exist. The 3-alkoxy-substituted 4-chloroisocoumarins and 4-chloro-7-nitroisocoumarins are simple acylating agents and do not give stable inactivated enzyme structures. Substituted isocoumarins are some of the most potent inactivators reported for many of the enzymes tested and may be quite useful as inhibitors of proteolysis both *in vivo* and *in vitro*.

Harper, J. W., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:7200-7213, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

VARIABILITY OF FUNCTIONAL CHARACTERISTICS OF MDCK CELLS

We measured several functional parameters of MDCK cells cultured as monolayers in order to more fully characterize their ion transport properties. Most of the present studies were completed with five groups (*A–E*) of MDCK cells studied from passage 62 to 78. Each group represents the same subline of MDCK cells after having been frozen, stored, and thawed at passage 62 or 64. The median transmonolayer resistances of the groups were 507, 149, 284, 72, and 126 $\Omega \cdot cm^2$. Addition of amphotericin B to the apical solution induced a ouabain-sensitive transepithelial current. The apical membrane voltage and fractional resistance exhibited a wide range of values in two of the groups studied, with mean values of -32 mV and 0.68 in *group B* and -40 mV and 0.78 in *group E*. Neither apical nor basolateral membrane displayed significant Na^+ conductance. K^+ conductance was present in the basolateral but not in the apical membrane. Acidification or alkalinization of the apical solution was dependent on the conditions used to study the cells. The 4,4′-diisothyocyano-2,2′-disulfonic stilbene inhibited acidification (or induced alkalinization), whereas increasing ambient HCO_3^- concentration induced alkalinization. The results of these studies indicate qualitatively similar behavior between five groups of MDCK cells but significant quantitative differences between the groups. Analysis of the variability of the measured parameters indicated that there were no differences as a function of passage number within a group. The factors responsible for functional differences between groups are not known but may be related to the cell storage process.

Husted, R. F., *Welsh, M. J.*, and Stokes, J. B.

American Journal of Physiology **250**:C214-C221, 1986.

Other support: National Heart, Lung and Blood Institute and the Cystic Fibrosis Foundation.

From the Laboratory of Epithelial Transport and Kidney Physiology, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

VI. *Immunology and Adaptive Mechanisms*

REGULATION OF THE Fc-RECEPTOR-MEDIATED RESPIRATORY BURST: TREATMENT OF PRIMED MURINE PERITONEAL MACROPHAGES WITH LIPOPOLYSACCHARIDE SELECTIVELY INHIBITS H_2O_2 SECRETION STIMULATED BY IMMUNE COMPLEXES

The effect of bacterial lipopolysaccharide on the Fc-receptor-mediated respiratory burst in murine peritoneal macrophages has been examined. After treatment overnight with small quantities of LPS, macrophages exhibited dramatic diminution of their capacity to generate and secrete H_2O_2 when triggered with immune complexes. The effect of LPS treatment was dependent on the state of macrophage functional activation; only cells that were primed or fully activated in vivo or were treated with interferon-γ in vitro were sensitive to this effect of LPS. The LPS-mediated loss of secretory function was both dose and time dependent and could be reproduced with the lipid A moiety of LPS. The effect was selective for H_2O_2 secretion triggered through the Fc receptor; the respiratory burst stimulated by phorbol diesters remained unaltered. Furthermore, LPS treatment did not alter either binding or ingestion of radiolabeled immune complexes in parallel with the change in H_2O_2 secretion, indicating that the suppressive effect was not due to compromised endocytic function. These results indicate that LPS treatment of primed macrophages regulates the function of Fc receptors and may uncouple receptor occupancy from generation and secretion of H_2O_2.

Johnston, P. A., *Adams, D. O.* and Hamilton, T. A.

The Journal of Immunology **135**(1):513-518, 1985.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

BIOCHEMICAL MODELS OF INTERFERON-γ-MEDIATED MACROPHAGE ACTIVATION: INDEPENDENT REGULATION OF LYMPHOCYTE FUNCTION ASSOCIATED ANTIGEN (LFA)-1 AND I-A ANTIGEN ON MURINE PERITONEAL MACROPHAGES

IFN-γ can induce the expression of both class II histocompatibility antigens (Ia) and the lymphocyte function associated (LFA)-1 antigen on murine peritoneal macrophages. We have examined the molecular changes which lead to altered expression of these two cell surface proteins to determine whether they are regulated by similar or independent mechanisms. While I-A antigen expression can be induced or enhanced by treatment of macrophages with either phorbol diesters and/or the Ca^{++} ionophore A23187, these agents had no effect upon expression of LFA-1 under similar conditions. Macrophages from the A/J strain mouse exhibit a deficiency in their sensitivity to IFN-γ which is seen in our studies as an inability of IFN-γ to elevate I-A antigen expression. However, expression of I-A could be modulated in these cells by treatment with either phorbol diesters or A23187. In contrast, IFN-γ could induce LFA-1 antigen on A/J

derived macrophages and this was not affected by either phorbol or A23187. Thus these two antigens, despite coordinate expression in response to IFN-γ in normal mouse strains, are clearly regulated independently. These results suggest that IFN-γ generates at least two independent molecular events in macrophages which ultimately modulate the expression of cell surface proteins important to the performance of activated functions.

Strassmann, G., Somers, S. D., Springer, T. A., *Adams, D. O.* and Hamilton, T. A.

Cellular Immunology **97**:110-120, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC, and the Laboratory for Membrane Immunochemistry, Dana Farber Cancer Center, Harvard University Medical Center, Boston.

LPS INDUCES ALTERED PHOSPHATE LABELING OF PROTEINS IN MURINE PERITONEAL MACROPHAGES

Covalent modification of proteins via phosphorylation is a well-documented mechanism whereby intracellular events are controlled by external stimuli. Treatment of thioglycollate-elicited, C57B1/6 murine peritoneal macrophages with nanogram quantities of bacterial lipopolysaccharide (LPS) consistently results in altered ^{32}Pi labeling of a specific set of proteins (*e.g.*, proteins of 67, 37, 33, and 28 kD), as measured by autoradiography after SDS-polyacrylamide gel electrophoresis. Induction of this pattern of phosphorylation is duplicated by the lipid A moiety of LPS. The LPS-stimulated changes in phosphate labeling are both dose- and time-dependent. The phosphorylation pattern induced in macrophages by the tumor promoter phorbol myristic acetate, one of various pharmacologic agents tested, shows similarity to the pattern induced by LPS. Analysis of pp 28 and pp 37 from both LPS- and PMA-treated macrophages by limited proteolysis demonstrates that these phosphoproteins are structurally related and that the sites of phosphorylation are similar for both treatment conditions. Macrophages from the genetically LPS-unresponsive C3H/HeJ strain show no alteration in their pattern of phosphorylation after treatment with LPS. Control macrophages, from C3H/HeN mice, respond to LPS in a fashion identical to that seen in C57B1/6 macrophages. Pretreatment of macrophages with IFN-γ potentiates the effect of LPS (*i.e.*, yields a level of altered phosphate labeling greater than observed with LPS or PMA alone). Together, the data indicate that LPS causes altered phosphate labeling of a defined set of proteins, and that the circumstances of this response are consistent with a possible role in coupling LPS-initiated signals to the induction of functional competence in macrophages.

Weiel, J. E., Hamilton, T. A. and *Adams, D. O.*

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Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

PHORBOL ESTERS AND CALCIUM IONOPHORE CAN PRIME MURINE PERITONEAL MACROPHAGES FOR TUMOR CELL DESTRUCTION

Murine macrophages from sites of inflammation develop toward tumoricidal competence by exposure to a macrophage-activating factor such as interferon-γ (IFN-γ). To explore the biochemical transductional events initiated by IFN-γ, peritoneal macrophages from C57BL/6J mice elicited by various sterile irritants were treated *in vitro* with two pharmacologic agents that mimic the action of certain second messengers. Phorbol myristate acetate (PMA) and the ionophore A23187 cooperatively reproduced the ability of IFN-γ to prime macrophages for tumoricidal function. Neither agent alone was able to prime macrophages. The two agents acted on the macrophages, and target susceptibility to kill was not altered by PMA and A23187. Only active phorbol esters, which are known to bind and stimulate protein kinase C, were able to cooperate with A23187 to induce priming. A cell-permeable synthetic diacylglycerol (sn-1,2-dioctanoyl glycerol) could also prime for cytolysis. In the presence of PMA, A23187, and EGTA, addition of Ca^{++} was sufficient for priming, whereas the addition of Mg^{++} was much less efficient. Priming by IFN-γ, however, was not blocked by EGTA. Efflux of $^{45}Ca^{++}$ from preloaded cells was significantly increased by A23187 and by IFN-γ. Quin-2/AM, an intracellular chelator of Ca^{++}, blocked priming by IFN-γ. In summary, the data suggest that priming of macrophages for tumoricidal function by IFN-γ involves, at least in part, alterations in protein kinase C and in levels of intracellular Ca^{++}.

Somers, S. D., Weiel, J. E., Hamilton, T. A., and *Adams, D. O.*

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From the Department of Microbiology/Immunology, Duke University Medical Center, Durham, NC.

EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE ON PROTEIN SYNTHESIS IN MURINE PERITONEAL MACROPHAGES: RELATIONSHIP TO ACTIVATION FOR MACROPHAGE TUMORICIDAL FUNCTION

Early biochemical events in the response of murine peritoneal macrophages to bacterial lipopolysaccharide (LPS) had been examined (i.e., 0-4 hr after initiation of treatment). At concentrations of 10 ng/ml or less, LPS stimulated the new or enhanced synthesis of a series of at least six polypeptides of 85, 80, 75, 65, 57, and 38 kD. This effect was dependent upon the lipid A moiety of LPS as lipid A itself could induce the changes and the effect of LPS could be blocked by inclusion of polymixin B sulfate in the culture medium. The effect was specific for LPS in that other endotoxin-free agents known to alter macrophage physiology could not produce the same changes. The time course of LPS stimulation of macrophage protein synthesis was remarkable in that the synthesis of all six proteins was transient even in the continued presence of LPS, being first detected approximately 1 hr after exposure and no longer apparent by 8-10 hr after treatment was initiated. Furthermore, both pulse-chase and cumulative radiolabeling studies indicated that at least two of the proteins (85 and 35 kD) were short-lived and did not accumulate in LPS-treated cells, suggesting the possibility that they participate in a regulatory rather than a functional role. Macrophage tumoricidal activation in-

volves cooperation in response to two independent signals; interferon gamma and LPS. Pretreatment of macrophages with interferon gamma increased the sensitivity of macrophages to LPS-stimulated protein synthesis by one to two orders of magnitude documenting such cooperativity in molecular terms. The LPS-induced stimulation of specific protein synthesis could be reproduced by treatment of macrophages with heat killed *Listeria monocytogenes*, a gram-positive, endotoxin-negative bacterial stain which has been shown to substitute effectively for LPS in macrophage tumoricidal activation. Furthermore, reversible inhibition (i.e., treatment with cycloheximide) of protein synthesis during LPS treatment abrogated the acquisition of tumoricidal function. These results identify an early biochemical response to LPS which may be a necessary component of the intracellular transduction of signals which regulate macrophage functional development.

Hamilton, T. A., Jansen, M. M., Somers, S. D., and *Adams, D. O.*

Journal of Cellular Physiology **128**:9-17, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

REGULATION OF RESPIRATORY BURST IN MURINE PERITONEAL MACROPHAGES: DIFFERENTIAL SENSITIVITY TO PHORBOL DIESTERS BY MACROPHAGES IN DIFFERENT STATES OF FUNCTIONAL ACTIVATION

Activation of macrophages either *in vivo* or *in vitro* can modulate the capacity to generate and secrete reactive oxygen intermediates including H_2O_2 and O_2^-. Thus, the cellular and biochemical components requisite for execution of the respiratory burst must be regulated during the activation process. In the present report, we have examined murine peritoneal macrophages in different stages of activation for their sensitivity to stimulants of respiratory burst known to activate protein kinase c (i.e., phorbol dibutyrate or diacylglycerol). The results demonstrated that more highly activated macrophages showed, in addition to greater magnitude of H_2O_2 or O_2^- production, a two- to fourfold greater sensitivity to these stimuli. While more active macrophages also exhibited a higher rate of H_2O_2 secretion, the time at which secretion was measured did not account for or modulate the heightened sensitivity. The increased sensitivity to stimulation was dependent upon the stage of activation and not on the agent used to elicit the macrophages. Increased sensitivity of the more active macrophage populations was also seen when physiologic stimuli (i.e., insoluble immune complexes or unopsonized zymosan) were used. These findings indicate that macrophage activation of H_2O_2 secretion modulates the sensitivity to stimulation such that more H_2O_2 is produced in a shorter time and at a lower concentration of stimulus, thereby heightening the inflammatory response in several independent ways. Because all the stimuli employed in the present study have in common the ability to activate protein kinase c (either directly or indirectly), the data also suggest that this form of macrophage activation may involve, at least in part, modulation of the stimulus-response coupling mechanism which utilize this enzyme.

Johnston, P. A., *Adams, D. O.*, and Hamilton, T. A.

Cellular Immunology **100**:400-410, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

TREATMENT OF MURINE PERITONEAL MACROPHAGES WITH BACTERIAL LIPOPOLYSACCHARIDE ALTERS EXPRESSION OF c-FOS AND c-MYC ONCOGENES

Expression of the c-fos, c-myc, and c-fms proto-oncogenes has been studied in thioglycollate-elicited murine peritoneal macrophages after exposure to lipopolysaccharide (LPS). After incubation with LPS (20 ng/ml), a transient and rapid induction of the expression of c-fos and c-myc oncogenes could be observed, whereas the RNA levels for c-fms were not affected. Treatment with lipid A, the active moiety of the LPS molecule, increased the c-fos and c-myc expression to a comparable degree. Similar induction of c-fos and c-myc was observed after treatment with phorbol myristate acetate, suggesting that this effect of LPS on murine macrophages might be mediated through stimulation of protein kinase C. Under similar experimental conditions, LPS treatment of macrophages did not trigger DNA synthesis. Treatment with LPS blocked DNA synthesis in macrophages treated with L cell-conditioned medium containing colony-stimulating factor. Thus changes in c-fos and c-myc expression may be elements in the complex series of biochemical events that contribute to macrophage activation and are not necessarily related to induction or priming for cellular proliferation.

Introna, M., Hamilton, T. A., Kaufman, R. E., and *Adams, D. O.*

The Journal of Immunology **137**(8):2711-2715, October 15, 1986.

From the Department of Pathology, Duke University Medical Center, Durham, NC.

ANALYSIS OF DEFICIENCIES IN IFN-γ-MEDIATED PRIMING FOR TUMOR CYTOTOXICITY IN PERITONEAL MACROPHAGES FROM A/J MICE

The functional and biochemical responses of macrophages derived from the A/J mouse strain to IFN-γ have been studied. As compared to macrophages obtained from C57BL/6 strain mice, cells from mice of the A/J strain are deficient in their response to IFN-γ for acquisition of tumoricidal competence. This deficiency was not due to reduced expression of surface receptors for IFN-γ or to altered affinity of the receptor for its ligand. IFN-γ recently has been shown to enhance the potential activity of protein kinase C (PKc) and to modulate the efflux of intracellular Ca^{++} in macrophages from C57BL/6 mice. Neither of these two biochemical changes was induced in macrophages derived from A/J mice. Functional competence could, however, be pharmacologically induced in both C57BL/6- and A/J-derived macrophages by combined treatment with an ionophore plus phorbol myristic acetate, which increase intracellular Ca^{++} and stimulate PKc, respectively. Although the exact nature of the deficit in A/J

strain mice has not been defined, the present findings indicate that it lies between the expression of receptor and the modulation of PKc activity and Ca^{++} levels. Furthermore, the data provide support for the notion that these molecular changes are important components of the stimulus-response coupling process in IFN-γ-mediated activation of macrophages.

Hamilton, T. A., Somers, S. D., Becton, D. L., and *Adams, D. O.*

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Other support: U. S. Public Health Service, Eli Lilly Research Laboratories and the Elsa V. Pardee Foundation.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

VIRAL INFECTION OF VASCULAR ENDOTHELIAL CELLS ALTERS PRODUCTION OF COLON-STIMULATING ACTIVITY

Viral infections in humans are frequently associated with granulocytopenia and/or granulocytosis. Such changes in myelopoiesis could result from infection of the granulocyte-macrophage colony-forming cell (CFC-GM) or changes in the production of colony-stimulating activity (CSA). Endothelial cells are a known source of CSA and may be transiently or persistently infected during a number of viral infections, including infection with herpes simplex virus type I (HSV-I) and measles virus. Therefore, we examined the effect of endothelial cell infection with these two viruses on the production of CSA. Uninfected passaged endothelial cells produce CSA when stimulated by the continual presence of a factor present in medium conditioned by peripheral blood monocytes (MCM). Within 4 h of infection with HSV-I, endothelial cells no longer produced CSA in response to MCM. In contrast, measles virus infection induced CSA production by passaged endothelial cells even in the absence of MCM. Measles virus-induced CSA production was maximal at 24 h and required the presence of live virus within the endothelial cells. The effects of HSV-I and measles virus on CSA production were not dependent on alterations in the production of α- or γ-interferon by the infected endothelial cells. Infection with HSV-I did not stimulate endothelial cells to release any detectable interferon. In contrast, the supernatants of the measles-infected cells contained only β-interferon, a known inhibitor of CFC-GM development. These studies suggest that CSA production by endothelial cells is directly altered by infection with HSV-I and measles virus. An alteration in CSA production might contribute to changes in myelopoiesis that frequently accompany viral infection in humans.

Gerson, S. L., Friedman, H. M., and *Cines, D. B.*

Journal of Clinical Investigation **76**:1382-1390, 1985.

Other support: National Institutes of Health and the Sohio Foundation.

From the Division of Hematology-Oncology, University Hospitals of Cleveland, Cleveland, OH; and the Divisions of Infectious Diseases and Hematology-Oncology, Hospital of the University of Pennsylvania, Philadelphia.

ANTIBODY AND IMMUNE COMPLEXES INDUCE TISSUE FACTOR PRODUCTION BY HUMAN ENDOTHELIAL CELLS

Patients with systemic lupus erythematosus (SLE) have an increased incidence of arterial and venous thromboses. The mechanism by which thromboses develop in these patients is unknown. We had previously observed that the sera of patients with SLE contain antibodies and immune complexes that can bind to endothelial cells. Because endothelial cells can synthesize tissue factor, a potent activator of coagulation, we studied the effect of IgG complexes and sera from patients with SLE on the production of tissue factor by these cells. Human umbilical venous endothelial cells incubated with heat-aggregated IgG (HA-IgG) (0.5 to 4.0 mg) elaborate procoagulant activity in a dose-dependent manner. All procoagulant activity was found in the particulate cell fraction, and none was secreted into the medium. Maximum expression of procoagulant activity required 6 to 8 hr, and its production was totally inhibited by the addition of cyclohexamide or actinomycin D. The presence of gel-filtered platelets augmented production of procoagulant activity by endothelial cells stimulated by HA-IgG. Endothelial cell procoagulant activity was not inactivated by diisofluoropropylphosphate, required the presence of Factor VII for its expression, and was neutralized by a specific anti-tissue factor antibody. Endothelial cells incubated with sera from 14 of 16 patients with SLE produced increased amounts of tissue factor compared with 21 normal sera ($p < 0.025$). Fractions of two SLE sera containing monomeric IgG, IgA, or IgM, as well as fractions containing IgG complexes, each stimulated endothelial cells to produce more tissue factor than similar fractions prepared from two normal sera. These studies demonstrate that endothelial cells will produce the procoagulant tissue factor after exposure to anti-endothelial cell antibodies or IgG-containing immune complexes. The production of tissue factor by endothelial cells at sites of immune vascular injury may play a role in the development of thromboses in patients with SLE.

Tannenbaum, S. H., Finko, R., and *Cines, D. B.*

The Journal of Immunology **137**(5):1532-1537, 1986.

Other support: National Institutes of Health.

From the Hematology-Oncology Section, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. V. CHARACTERIZATION OF My-10 ANTIGEN EXPRESSION BY NORMAL LYMPHOHEMATOPOIETIC PROGENITOR

The My-10 glycoprotein is an hematopoietic cell surface antigen expressed specifically by undifferentiated (blast) cells, constituting 1%–4% of normal adult bone marrow leukocytes. We used several immunological and in vitro culture methods to analyze the expression of this unique antigen on a variety of lymphohematopoietic progenitor cells. Colony-forming cells (CFC) for granulocyte-monocyte colonies (CFC-GM) and erythroid colonies (BFU-E) were predominantly My-10 positive. CFC with higher proliferative potential were more strongly My-10 positive than CFC with lower proliferative potential, and those for mixed-lineage and blast cell colonies were even more uniformly My-10 positive. Cells maintaining CFC-GM number in short-term marrow culture (pre-CFC) were found to be My-10 positive, as were lymphoid

precursors defined by their content of intranuclear terminal deoxynucleotidyl transferase. More mature erythroid precursors (CFU-E) were heterogeneous for antigen expression and lost My-10 antigen progressively, in parallel with advancing maturational stage. The My-10 antigen permits rapid identification and purification of hematopoietic progenitor cells for further study or potential clinical application. The disappearance of the My-10 antigen, moreover, may be a probe for differentiation-linked cellular events.

Strauss, L. C., Rowley, S. D., La Russo, V. F., Sharkis, S. J., Stuart, R. K., and *Civin, C. I.*

Experimental Hematology **14**:878-886, 1986.

Other support: National Institutes of Health and the American Cancer Society.

From The Johns Hopkins Medical Center, Divisions of Pediatric Oncology and Experimental Hematology, Johns Hopkins University School of Medicine, Baltimore, MD.

SIALYLATED GLYCOLIPID ANTIGENS ON HUMAN LEUKEMIC CELL LINES

Many granulocyte-specific mouse monoclonal antibodies recognize the carbohydrate sequence 3-fucosyllactosamine, Galβ1–4[Fucα1–3]GlcNAc, which occurs in cell-surface glycolipids and glycoproteins. In general, these antibodies bind to blast cells from most patients with acute myeloblastic leukemia, but not to those with acute lymphocytic leukemia. Neuraminidase treatment, however, increases exposure of this antigen on both myeloid and lymphoid cells. In the present study, the glycolipids from 13 lymphoid and nonlymphoid human cell lines were examined for the presence of unsialylated and sialylated 3-fucosyllactosamine sequences using a thin-layer chromatography immunostaining method. Nine of the cell lines were also tested by indirect immunofluorescence both before and after neuraminidase treatment. None of the six B-cell and T-cell lines had detectable neutral or sialylated glycolipid antigen. In contrast, six out of seven and five out of seven nonlymphoid cell lines had neutral and sialylated glycolipid antigens, respectively. These results agreed, in general, with those found by indirect immunofluorescence. They also represent the first direct demonstration of these sialylated glycolipids on human leukemic cells. Thus, in some cases increased antibody binding to neuraminidase-treated cells can be explained by the presence of sialylated glycolipid antigen.

Spitalnik, S. L., Spitalnik, P. F., *Civin, C. I.*, Ball, E. D., Schwartz, J. F., and Ginsburg, V.

Experimental Hematology **14**:643-647, 1986.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; the Division of Pediatric Oncology, The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, MD; and the Department of Medicine, Dartmouth Medical School, Hanover, NH.

IN VITRO RELEASE OF α_1-ACID GLYCOPROTEIN RNA SEQUENCES SHOWS FIDELITY WITH THE ACUTE PHASE RESPONSE *IN VIVO*

The acute phase reaction of rat liver to subcutaneous turpentine challenge results in a 20- to 100-fold increase in α_1-acid glycoprotein (αAGP) mRNA. We utilized this response to establish conditions appropriate for study of RNA transport *in vitro* using hybridization with ^{32}P-labeled exon and intron αAGP sequences. Contamination of nuclear preparations by membrane-absorbed cytoplasmic RNA was eliminated by detergent-rinsing. The *in vitro* incubation conditions that most reflected the *in vivo* state required RNase inhibitor (purified from placenta), polyvinylpyrrolidone to prevent nuclear swelling, and addition of ATP. Under these circumstances, αAGP sequences were transported only from turpentine-stimulated preparations, were found only in poly(A)+ RNA, and were the same size as authentic cytoplasmic mRNA. Omission of polyvinylpyrrolidone resulted in release of some αAGP sequences in smaller, more heterogeneous poly(A) RNA, and leakage of some αAGP sequences was observed from control preparations. Omission of ATP resulted in restriction of mature αAGP mRNA to the nucleus. In contrast to αAGP mRNA, transport of albumin mRNA was decreased 3-4X in turpentine-treated preparations. The largest αAGP intron was not found in RNA transported from treated nuclei in complete medium. The intron-containing fragments remained in the nucleus, largely in poly(A) RNA of a size consistent with free intron. Some hybridization of intron sequences was observed with cytoplasmic and nuclear membrane-associated poly(A)+ RNA preparations which may represent 3′-processing catabolites; leakage of these sequences was considerably greater in the absence of PVP. On the basis of densitometric estimates, a 5-fold increase in the amount of αAGP exon sequences was observed in nuclear RNA, comparing treated with control animals, but transport of αAGP exon sequences was detectable only from treated nuclei, indicating at least a 50-fold increase in abundance of αAGP sequences. This suggests that a selective gating mechanism may be operative at the level of posttranscriptional nucleocytoplasmic transport during induction of αAGP in the acute phase response.

Clawson, G. A. et al.

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Other support: National Institutes of Health and the Academic Senate, University of California at San Francisco.

From the Department of Pathology, University of California School of Medicine, San Francisco.

JH$_1$ PEPTIDE INDUCES ANTIBODIES TO A COMMON IMMUNOGLOBULIN DETERMINANT AS DETECTED BY CELL-BINDING ANALYSIS

In order to more accurately determine the distribution of antigenic determinants detected by antisera to hypervariable-region and JH$_1$ peptides, we measured the frequency of lymphocytes stained with these sera by flow cytometry. None of the sera specific for HV1, HV2 or HV3 peptides stained significant numbers of lymphocytes, but those specific for JH$_1$ reacted with nearly all B-cells.

Seiden, M. V., Srouji, A., *Clevinger, B.*, and Davie, J. M.

Molecular Immunology **23**(2):125-129, 1986.

Other support: U. S. Public Health Service.

From the Department of Microbiology and Immunology, School of Medicine, and Division of Biomedical Sciences, School of Dental Medicine, Washington University, St. Louis.

EFFECT OF HUMAN SERUM AND SOME OF ITS COMPONENTS ON NEUTROPHIL ADHERENCE AND MIGRATION ACROSS AN EPITHELIUM

The effect of human serum and some of its components on the process of transepithelial migration of human neutrophils was investigated in an *in vitro* system. 10% autologous serum caused an increase in neutrophil adherence to and migration across canine kidney epithelial cells. This increase in neutrophil binding also occurred if the epithelium but not the neutrophils had been preincubated with serum. The binding was lost if the serum was either preabsorbed over the kidney epithelium before use or heat inactivated. Indirect immunofluorescence studies indicated that IgG, IgM, and a component of C3 bound to the epithelial surface, whereas IgA, IgE, or C5a were not detectable. The majority of epithelial cells were immunofluorescent, however epithelial cells with varying degrees of reactivity were also apparent and ~5% of the epithelial cells did not bind IgG, IgM, and C3. When epithelia were simultaneously tested for the presence of either IgG, IgM, or C3, and bound neutrophils, the few epithelial cells which did not bind IgG or IgM also did not bind C3 or neutrophils. Studies with monoclonal antibodies against Fc and C3 receptors indicate that neutrophil adherence to the epithelial surface was mediated predominately by the receptors for C3b and C3bi. In response to a chemotactic gradient, bound neutrophils were able to detach and migrate across the epithelium. A separate heat-stable factor(s) in serum was able to increase neutrophil migration across the epithelial monolayer. This factor acted independently of the factors which caused the increase in neutrophil binding as the increase in neutrophil migration also occurred under conditions (preabsorption over the kidney epithelium or heat inactivation) that prevented the increase in neutrophil binding. The increase in neutrophil migration may be caused by the permeability-increasing properties of this factor as both serum and heat-inactivated serum lowered the transepithelial electrical resistance an average of 38 and 35%, respectively, in 40 min. Upon removal of serum or heat-inactivated serum, the resistance returned 100 and 81%, respectively, in 5 h.

Cramer, E. B., et al.

The Journal of Cell Biology **102:**1868-1877, 1986.

Other support: National Institutes of Health and the New York Heart Association.

From the Department of Anatomy and Cell Biology, State University of New York, Downstate Medical Center, Brooklyn.

ALL HUMAN MONOCYTES HAVE THE CAPABILITY OF EXPRESSING HLA-DQ AND HLA-DP MOLECULES UPON STIMULATION WITH INTERFERON-γ

We have simultaneously studied expression of all three classes of human Ia (HLA-DR, DP, and DQ) on normal human B cells and monocytes by using two-color

immunofluorescence and flow cytometry. Expression was investigated on freshly isolated cells and after incubation of cells for 48 and 96 hr in interferon-γ (IFN-γ). All freshly isolated B cells express high levels of DR, DQ, and DP, and these levels are unchanged by incubation with IFN-γ for 48 hr and 96 hr. In contrast, freshly isolated monocytes are on the average 91% DR^+, 32% DQ^+, and 15% DP^+. Incubation with IFN-γ increases Ia expression on Mϕ to 98% DR^+, 75% DQ^+, and 58% DP^+ at 48 hr, with virtually all cells becoming positive for all three Ia antigens at 96 hr. Furthermore, after the 96-hr incubation, antigen density increases 10-fold for DR, 15-fold for DQ, and 15-fold for DP in monocytes to reach levels of expression comparable with B cells. These studies demonstrate that all peripheral blood monocytes have the capacity to become HLA-DQ and HLA-DP positive; IFN-γ regulates expression of all three classes of human Ia in monocytes; and IFN-γ does not significantly modulate Ia expression in B cells.

Gonwa, T. A., Frost, J. P. and *Karr, R. W.*

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Other support: The Veterans Administration.

From the Veterans Administration Medical Center and the Department of Internal Medicine, University of Iowa, Iowa City.

GAMMA INTERFERON AND 5-AZACYTIDINE CAUSE TRANSCRIPTIONAL ELEVATION OF CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX GENE EXPRESSION IN K562 LEUKEMIA CELLS IN THE ABSENCE OF DIFFERENTIATION

We studied the effects of gamma interferon (IFN-γ) on HLA class I gene expression, differentiation, and proliferative capacity of K562 human leukemia cells. In the uninduced state, K562 cells show little or no class I gene expression but actively express the erythroid-specific γ-globin gene as well as genes associated with cell proliferation, including the transferrin receptor, c-*myc*, and α-actin genes. At both the surface protein and mRNA levels, IFN-γ induces class I and β_2-microglobulin gene expression, but does not alter the expression of the γ-globin, transferrin receptor, c-*myc*, or α-actin genes. A 10-fold maximal induction of both class I surface protein and mRNA occurs at 48 h and is reversible upon withdrawal of IFN-γ from the culture medium. In vitro nuclear run-on transcription assays were performed to directly establish that IFN-γ exerts an early effect at the level of transcription, with maximal transcription rates occurring within 4 h. The difference between the time course of transcription induction and that of mRNA accumulation suggests that the regulation of class I gene expression in this human leukemic cell line also involves posttranscriptional mechanisms. Measurements of cell proliferation rates and cell cycle distribution, as well as the reversibility of the effects of IFN-γ, demonstrate that the selective induction of class I genes in these cells occurs in the absence of differentiation.

Chen, E., *Karr, R. W.*, Frost, J. P., Gonwa, T. A., and Ginder, G. D.

Molecular and Cellular Biology **6**(5):1698-1705, 1986.

Other support: National Institutes of Health.

From the Veterans Administration Medical Center and Department of Internal Medicine, University of Iowa College of Medicine, and Genetics Ph.D. Program, University of Iowa, Iowa City.

TOWARDS A UNIFIED THEORY OF IMMUNOGLOBULIN STRUCTURE-FUNCTION RELATIONS

The network theory (Jerne 1974) of immune regulation requires selection of binding sites against binding sites. It is difficult to reconcile this axiom with the concept of a series of antibodies in which the binding sites are all composed of interacting residues recessed in a pocket. In this review we address the structural and functional specialization of the antibody molecule from the point of view that there is overlap between idiotypic and paratopic repertoires. This overlap leads to the introduction of multipotentiality at the level of the immunoglobulin itself while still adhering to the clonal selection theory (Burnet 1959). The possible absence of distinction between CDR and idiotope determining regions (IDR) suggests a unified concept of Ig structure which interacts with antigens and idiotopes in the immune network. We call this concept the Variable Surface Recognition Model. Herein we draw upon information about the antigenic structure of proteins, the biological response to these antigenic structures, structural principles to which proteins adhere, anti-idiotypic and synthetic peptide experiments and the structural characteristics of immunoglobulins to justify such a unified concept.

Kieber-Emmons, T. and *Kohler, H.*

Immunological Reviews **90**:29-48, 1986.

Other support: American Cancer Society and the National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

IMMUNOGLOBULIN WITH COMPLEMENTARY PARATOPE AND IDIOTOPE

A hybridoma antibody (11E7-1) was isolated from a myeloma fusion with *nu/nu* BALB/c immunized against the T15 idiotype. This IgM antibody exhibited a dual specificity, binding both to PC and to anti-PC antibodies from two idiotype families. Binding to PC and anti-PC antibodies are completely inhibited by PC analogs. Furthermore, the hybridoma antibody binds to itself. Self-binding is also inhibited by PC analogs. From these data, we suggest that 11E7-1 hybridoma antibody has a PC-specific paratope site, and at the same time expresses the internal PC antigen idiotope. The term autobody is proposed to signify its self-binding and potential role in autoimmunity. Autobodies may have a unique role in the network of immune system. Furthermore, it may be a model for designing idiotype vaccines.

Kang, C.-Y. and *Kohler, H.*

Journal of Experimental Medicine **163**:787-796, 1986.

Other support: American Cancer Society and the National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

IDIOTOPE ANTIGENS (Ab2α AND Ab2β) CAN INDUCE IN VITRO B CELL PROLIFERATION AND ANTIBODY PRODUCTION

We previously showed that immunization of various strains of mice with three types of antigen—PC-Hy (nominal antigen), F6-Hy (Ab2α-Hy), and 4C11-Hy (Ab2β-Hy)—induces a differential PC-specific, T15-Id⁺ antibody response. In this report, the in vitro phosphorylcholine (PC)-specific B cell responses induced by these three antigens were studied. A hemocyanin-specific long-term T helper cell line was used to provide help for primary and secondary in vitro T cell-dependent B cell responses. At low doses (0.005 to 0.5 μg/ml) of antigen, a significant increase in the proliferation of PC-OVA-primed BALB/c B cells was observed with Ab2-Hy or PC-Hy conjugate, but not unconjugate, antigens. Similar low doses of antigen could stimulate naive B cells to secrete IgM and stimulate PC-OVA- or 4C11-Hy-primed B cells to secret IgM and IgG1 anti-PC antibodies. The percentage of T15-Id of the PC-specific antibodies produced in the in vitro T-B culture was found to be less dominant than that produced by in vivo immunization, suggesting that certain regulatory mechanisms occur in the in vivo environment that may help to maintain the T15-Id dominance. Taken together, our in vivo and in vitro results indicate that idiotope antigens can function like nominal antigens to induce antigen-specific B cell responses. The mechanisms of thymic-dependent B cell activation induced by idiotope and nominal antigen are similar in that the T-B interaction is MHC-restricted and requires cognate recognition.

Huang, J.-H., Ward, R. E. and *Kohler, H.*

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Other support: National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

TUMOR-SPECIFIC IDIOTYPE VACCINES: I. GENERATION AND CHARACTERIZATION OF INTERNAL IMAGE TUMOR ANTIGEN

The concept of idiotype vaccines against tumor-associated antigens (TAA) was tested in the DBA/2 L1210 lymphoma subline, L1210/GZL. Monoclonal antibodies against a TAA that cross-reacts with the envelope glycoprotein gp52 of the mammary tumor virus were used to make hybridoma anti-idiotype antibodies (Ab2). In this report we describe the characterization of monoclonal anti-idiotypic antibodies against the combining site of 11C1 (Ab1), which recognizes a shared determinant of gp52 of mouse mammary tumor virus (MMTV) and the TAA of L1210/GZL. Hybridomas expressing the internal image of gp52 were screened by an idiotype inhibition assay. Mice sensitized with radiated L1210/GZL cells produced specific delayed type hypersensitivity (DTH) against the Ab2 hybridoma. Five Ab2 hybridomas were selected and were used to immunize DBA/2 mice. Such immunized animals showed specific DTH reaction against a challenge with the L1210/GZL tumor cells. Similar results were obtained in mice immunized with purified Ab2. Fluorescence-activated cell sorter analysis demonstrated that fluorescence staining of L1210/GZL cells by 11C1 can be completely inhibited with preabsorption on Ab2 hybridoma cells. Mice immunized with 2F10 and 3A4 coupled to keyhole limpet hemocyanin (KLH) contained antibodies binding to MMTV. But only in mice immunized with 2F10-KLH was significant inhibition of L1210/GZL tumor growth observed. Collectively, these results indicate that certain

anti-idiotypic antibodies can mimic the MMTV gp52 antigen, as well as the gp52-like epitope expressed on the L1210 GZL tumor cells. These properties of anti-idiotypic antibodies mimicking TAA could be exploited for making idiotype vaccines against tumors.

Raychaudhuri, S., Saeki, Y., Fuji, H., and *Kohler, H.*

The Journal of Immunology **137**(5):1743-1749, 1986.

Other support: American Cancer Society and National Cancer Institute.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

CIGARETTE SMOKING AND BRONCHOALVEOLAR T-CELL POPULATIONS IN SARCOIDOSIS

Pulmonary physicians must often deal with patients, including patients with sarcoidosis, who smoke cigarettes. Since changes in local pulmonary immune function have been associated with both sarcoidosis and cigarette smoking, it is important to distinguish which of these immunological changes in the lungs are due to the disease, which are due to cigarette smoking, and which, perhaps, are due to both.

Abnormally large numbers of helper thymus-derived (T) lymphocytes are found in fluids recovered by bronchoalveolar lavage (BAL) from patients with sarcoidosis. By contrast, normal numbers of lymphocytes are found in BAL fluids from normal cigarette smokers, and lower than normal percentages of the cells in these fluids are lymphocytes. The effects of smoking on T lymphocyte subpopulations in normal cigarette smokers and on those in patients with sarcoidosis, however, have not been thoroughly characterized.

The purposes of this study, therefore, were 1) to determine the effects of smoking on T lymphocyte subpopulations in BAL fluids from healthy normal volunteers (''normals'') and on those in BAL fluids from patients with sarcoidosis (''sarcoids''), and 2) to determine whether comparisons of the T lymphocyte subpopulations of normals and those of sarcoids revealed any effects of cigarette smoking.

Lawrence, E. C. et al.

Annals of the New York Academy of Sciences **465**:657-664, June 6, 1986.

Other support: National Institutes of Health and the General Clinical Research Center.

From the Department of Medicine, Baylor College of Medicine, Houston.

IMMUNOCHEMICAL AND PHARMACOLOGICAL DISTINCTIONS BETWEEN CURAREMIMETIC NEUROTOXIN BINDING SITES OF CENTRAL AUTONOMIC, AND PERIPHERAL ORIGIN

Comparative pharmacological and immunochemical studies were conducted on α-bungarotoxin binding sites from rat brain or muscle, *Torpedo* electric tissue, or the TE671 or PC12 clonal cell lines. Characteristic distinctions were observed in the pharmacological profile of drugs competing for toxin binding to different tissues. Differences also were found in the proportion of toxin binding sites (membrane-bound

or detergent-solubilized) that are immunologically reactive with either monoclonal antibodies directed against nicotinic acetylcholine receptors from the electric organ of *Torpedo* or polyclonal antisera raised against nicotinic receptors from the electric organ of *Electrophorus*. These results suggest that toxin binding sites are structurally heterogeneous. Structural heterogeneity of nicotinic acetylcholine receptors, neurotoxin binding sites, or both, may contribute to the manifestation of nicotinic receptor functional heterogeneity and may explain the apparent discrepancy at some sites between toxin binding activity and toxin functional potency.

Lukas, R. J.

Proceedings of the National Academy of Sciences, USA **83**:5741-5745, August 1986.

Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, and the Men's and Women's Boards of the Barrow Neruological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA*: II. THE ROLE OF MACROPHAGES AND T CELL KILLING

T lymphocytes from immune mice can adoptively transfer protection against infection with the extracellular Gram-negative bacterium *Pseudomonas aeruginosa* to nonimmune recipients, and in vitro, immune T cells are able to kill these bacteria. Earlier studies indicated that this killing is mediated by a bactericidal lymphokine. Those studies also showed that macrophages enhance this in vitro T cell killing but do not directly participate in the bacterial killing, nor do macrophages function to present antigen to T cells. The current studies demonstrate that the ability of macrophages to enhance T cell killing can be replaced by macrophage culture supernatants or by purified recombinant interleukin 1 (IL 1). In addition, the macrophage supernatant-induced enhancement can also be blocked by antibody to purified IL 1. These studies also demonstrate that the T cell subset that serves as the final effector cell in the killing process is the Lyt-1$^-$, 2,3$^+$, I-J$^+$ phenotype.

Markham, R. B., et al.

The Journal of Immunology **134**(6):4112-4117, June 1985.

Other support: National Institutes of Health and the National Science Foundation.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA* III. THE ROLE OF SUPPRESSOR T CELLS IN NONRESPONDER MICE

T lymphocytes from immune BALB/c mice can adoptively transfer protection against infection with the extracellular Gram-negative bacterium *Pseudomonas aeruginosa* to nonimmune recipients, and *in vitro*, immune T cells are able to kill these bacteria. Earlier studies indicated that this killing is mediated by a bactericidal lympho-

kine. The current studies demonstrate that T cells from immunized CB.20 mice, a strain congenic with BALB/c, fail to kill *Pseudomonas aeruginosa* in vitro. This nonresponsiveness is attributable to the activity of suppressor T cells of the Lyt-1$^+$, 2,3$^+$, I-J$^+$ phenotype. CB.20 mice are known to differ from BALB/c mice only at a single locus, which includes the Igh-1 allotype C_μ genes. These results suggest a critical role for this locus or closely linked genes in the control of T cell killing of this extracellular bacterium.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

The Journal of Immunology **136**(1):299-303, January 1, 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

T LYMPHOCYTE-MEDIATED PROTECTION AGAINST *PSEUDOMONAS AERUGINOSA* INFECTION IN GRANULOCYTOPENIC MICE

BALB/c mice immunized with *Pseudomonas aeruginosa* immunotype 1 polysaccharide develop protective T cell immunity to bacterial challenge. *In vitro*, T cells from immunized mice kill *P. aeruginosa* by production of a bactericidal lymphokine. The present study demonstrates that adoptive transfer of T cells from immunized BALB/c mice to granulocytopenic mice resulted in 97% survival on challenge with *P. aeruginosa*, compared with 17% survival with adoptive transfer of T cells from nonimmune BALB/c mice. This protection is specifically elicited by reexposure to the original immunizing antigen; adoptive recipients cannot withstand challenge with immunotype 3 *P. aeruginosa*. However, the adoptive recipients do survive simultaneous infection with both *P. aeruginosa* immunotypes 1 and 3. Adoptive transfer of T cells from the congenic CB.20 mice, which are unable to kill *P. aeruginosa* in vitro, provides only 20% protection to granulocytopenic mice. These studies indicate that transfer of specific immune T lymphocytes can significantly enhance the resistance to *P. aeruginosa* infection in granulocytopenic mice.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

Journal of Clinical Investigation **78**:375-380, August 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA*: IV. NONRESPONSIVENESS IN POLYSACCHARIDE-IMMUNIZED BALB/c MICE IS ATTRIBUTABLE TO VINBLASTINE-SENSITIVE SUPPRESSOR T CELLS

We reported previously that BALB/c mice immunized with a polysaccharide (PS) antigen isolated from immunotype 1 *Pseudomonas aeruginosa* and vinblastine

sulfate develop T cell-mediated protective immunity, despite their failure to produce specific antibody. *In vitro*, Lyt-1$^-$,2$^-$, I-J$^+$ T cells from vinblastine- and PS-immunized mice kill *P. aeruginosa* by secretion of a bactericidal lymphokine. BALB/c mice immunized with PS alone generate neither protective antibodies nor a protective T cell response. The current studies indicate that T cells from mice immunized with PS alone significantly suppress the bactericidal activity of T cells from mice immunized with vinblastine and PS. The suppressor T cells are of the same Lyt-1$^-$,2$^-$, I-J$^+$ phenotype as the bactericidal T cells. Suppression is mediated by a soluble product of these suppressor T cells which both inhibits T cell proliferation and interferes with the production or release of the bactericidal lymphokine. Cyclophosphamide, used in other systems to remove suppressor T cells, fails to enhance bacterial killing and does not inhibit suppressor cell activity. These studies indicate that immunization with PS elicits responses in two functionally distinct subgroups of Lyt-1$^-$,2$^-$, I-J$^+$ T cells, and that these cells are distinguishable by their sensitivity to vinblastine sulfate.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

The Journal of Immunology **137**(6):2025-2030, September 15, 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

MUTUAL RELATIONSHIP AMONG CYTOSOLIC pH, Na^+ AND Ca^{++} IONS IN THE DEGRANULATION OF RAT LEUKEMIC BASOPHILS

Reagents which affect the cytosolic concentrations of protons and sodium ions markedly affect the degranulation process of mast cells. The proton–sodium exchanging ionophore, monensin, is found to cause noncytolytic dose dependent serotonin release from the rat leukemic basophils (line RBL-2H3). Its half maximal dose of ca. 2 μM leads to secretion of ca. 20% of these cells' serotonin content. Monensin induced serotonin secretion increases with external pH and decreases upon lowering external sodium ion concentrations, yet is independent on external calcium. Monitoring cytosolic pH and free Ca^{++} concentrations with BCECF and quin2, respectively, shows that a rise in pH$_i$ and [Ca^{++}]$_i$ is caused by the ionophore. Amiloride, the blocker of cellular Na^+/K^+ antiporter, is found to be an effective inhibitor of antigen or monensin induced serotonin release. However, it does not by itself cause secretion. In contrast, ouabain, which inhibits the cellular Na^+/K^+ ATPase, does induce secretion. Cellular levels of pH$_i$, Na^+ and Ca^{++} ions are evidently linked and involve a manifold of activities. Though exchanging protons for sodium seems to be effective in causing mediator release, the present results do not provide sufficient support for proton/sodium ions having a second messenger role in the immunologically induced mediator release.

Sussman, Y., Reck, B. and *Pecht, I.*

Immunology Letters **13**:215-219, 1986.

From the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

IMMUNOLOGIC PROPERTIES OF ENDOTHELIAL CELLS

Endothelial seeding of vascular grafts has been shown to reduce thrombogenicity, improve patency rates, and improve graft incorporation. However, endothelial cells possess the major histocompatibility antigens, and antibodies directed against endothelium have been associated with graft rejection. At present, the best approach seems to be to avoid anti-donor immunologic reactivity by seeding autologous endothelial cells onto grafts prior to implantation. The surface of endothelial cells normally is actively antithrombogenic by virtue of the cells' abilities to synthesize and release PGI_2, and because of a surface ADPase. However, endothelium that is injured by viral infection, bacterial endotoxin, white blood cell lysates, or antibodies to endothelial surface antigens in the presence of complement, can bind immune complexes and can support complement-linked and procoagulant activities leading to vascular occlusion and damage. Nevertheless, the advantages of an endothelial lining are such that efforts should be made to understand better the conditions that may lead to endothelial dysfunction.

Ryan, U. S.

Asaio 8(2):58-64, April-June 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ISOLATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES REACTIVE WITH ENDOTHELIAL CELLS

Monoclonal antibodies were generated to antigens on cultured human umbilical vein endothelial cells. Spleen cells from BALB/c mice, immunized with low passage cultures of human umbilical vein endothelial cells, were fused with the non-secretory myeloma line, P3 × 63Ag 8.653. Hybridoma supernatants were screened for the desired immunological reactivity using ELISA binding assays. Hybridomas secreting antibodies reacting with the immunizing endothelial cells, but not with peripheral blood mononuclear cells, were cloned by limiting dilution and three stable clones were chosen for study. Further testing by ELISA revealed that each antibody displayed a unique pattern of reactivity. One antibody, 14E5, reacted with the macrophage-like cell line DHL-2, cultured macrophages derived from peripheral blood monocytes, and macrophages derived from malignant effusions. The antibody failed to react with fibroblasts or bovine endothelial cells. The second antibody, 12C6, reacted with human and primate fibroblasts and endothelial cells derived from bovine arteries, but not with mature macrophages. The third clone, 10B9, reacted only with immunizing endothelial cells and the immature-macrophage line U-937. All three antibodies failed to react with long-term human B or T lymphoblastoid cell lines, leukemic cell lines, or murine macrophage lines. None of the antibodies reacted with a battery of human epithelial derived cell lines or primary cultures of human epithelial cells. Indirect immunofluorescence assays revealed that the antigens were expressed on the cell surface. These antibodies should prove useful as differentiation markers of human endothelial cells and in studies of endothelial cell function.

Hamburger, A. W., Reid, Y. A., *Ryan, U.* and *Cines, D. B.*

Tissue & Cell **17**(4):451-459, 1985.

Other support: National Institutes of Allergy and Infectious Disease and a Research Career Development Award.

From the Cell Culture Department, American Type Culture Collection, Rockville, MD.

REGULATION OF T CELL AUTOCRINE GROWTH

During the course of investigating the regulation of IL-2-dependent T cell proliferation, we found that the subset of human T cells expressing the T4 surface glycoprotein become refractory to IL-2 growth promotion earlier than $T8^+$ cells. Since $T4^+$ cells proliferate in an autocrine fashion to endogenous IL-2, whereas most $T8^+$ cells respond in a paracrine fashion to IL-2 derived from $T4^+$ cells, we thought it likely that a unique mechanism was operative to restrict $T4^+$ cell IL-2-dependent autocrine proliferation. Moreover, we anticipated that the $T4^+$ cell IL-2-refractory state related either to suppression by $T8^+$ cells, or to expression of $T4^+$ cell IL-2-R. However, several experimental approaches did not support either of these mechanisms as being responsible for the loss of $T4^+$ cell IL-2 responsiveness. Isolated $T4^+$ cells ceased to respond to IL-2 well before $T8^+$ cells, and before the disappearance of adequate levels of IL-2-R. Moreover, a detailed comparison of IL-2-R expression by $T4^+$ vs. $T8^+$ cells revealed no differences in the number, affinity, rate of expression, or functional activity of high-affinity IL-2-R expressed by the two subsets. Accordingly, $T4^+$ cell autocrine IL-2 responsiveness is restricted by a mechanism that is independent of IL-2-R, and which ultimately results in cessation of both $T4^+$ and $T8^+$ cell IL-2-dependent clonal expansion.

Gullberg, M. and *Smith, K. A.*

Journal of Experimental Medicine **163**:270-284, February 1986.

Other support: National Cancer Institute and the Eli Lilly Corporation.

From the Department of Medicine, Dartmouth Medical School, Hanover, NH.

ANTIBODIES SPECIFIC FOR THE Mac-1, LFA-1, p150,95 GLYCOPROTEINS OR THEIR FAMILY, OR FOR OTHER GRANULOCYTE PROTEINS

In this report from the 2nd International Workshop on Human Leukocyte Differentiation Antigens, the authors note that a family of functionally important leukocyte surface glycoproteins which share a common β subunit of M_r = 95,000 has recently been defined in humans and mice. These glycoproteins, the lymphocyte function-associated 1 (LFA-1), macrophage 1 (Mac-1), and p150,95 molecules, each contain a different α subunit noncovalently associated with the common β subunit in an α,β structure. Monoclonal antibodies specific for the LFA-1 and Mac-1 molecules have allowed definition of their cell distribution and functions.

The LFA-1 molecule is expressed on B and T lymphocytes, NK cells, monocytes, and granulocytes. Monoclonal antibodies to LFA-1 block cytolytic T lymphocyte-mediated killing, natural killing and T helper cell responses. The Mac-1 molecule, identical to OKM1 and Mol1, is expressed on granulocytes, monocytes, and natural killer cells, and is absent on lymphocytes. The third member of the family, p150,95, named after the $M_r \times 10^3$ of its subunits, has been defined biochemically after isolation from myeloid cells with anti-β mAb.

In this study, the 118 mAbs submitted in the myeloid panel of the Second International Conference on Human Leukocyte Differentiation Antigens were tested for reactivity with members of the Mac-1, LFA-1, p150-95 family. It was of interest to compare mAbs from different laboratories, which have been the subject of several publications. Furthermore, the following questions were addressed: (1) Which mAbs reacted with specific members of the family and which cross-reacted with all three members? did any show unusual specificities, such as cross-reaction between only two members of the family? (2) Could mAbs specific for the p150,95 molecule be identified in the panel? (3) Which members of the family were up-regulated on myeloid cell surfaces by chemo-attractants? (4) Which mAbs in the myeloid panel were negative on Mac-1, LFA-1-deficient patients? How specific was the deficiency to the Mac-1, LFA-1 deficient patients? How specific was the deficiency to the Mac-1 LFA-1, p150-95 glycoprotein family?

In the course of these studies, information was also obtained on molecules distinct from Mac-1, LFA-1, and p150,95. This is presented as an appendix.

Springer, T. A. and Anderson, D. C.

In: Reinherz, E., Haynes, B., Nadler, L., Bernstein I. (eds.): *Leukocyte Typing II: Volume 3, Human Myeloid and Hematopoietic Cells*, New York: Springer-Verlag, 1986, Chap. 4, pp. 55-68.

Other support: National Institutes of Health.

From the Harvard Medical School, Boston.

MECHANISMS OF TUMOR CELL CAPTURE BY ACTIVATED MACROPHAGES: EVIDENCE FOR INVOLVEMENT OF LYMPHOCYTE FUNCTION-ASSOCIATED (LFA)-1 ANTIGEN

The lymphocyte function-associated (LFA)-1 molecule is expressed on certain populations of macrophages that have an augmented capacity to capture tumor cells. Accordingly, we analyzed the role of LFA-1 in the establishment of such cell-cell interactions. F(ab')$_2$ fragments of the M17/4, anti-LFA-1 monoclonal antibody (MAb) inhibited the interaction between activated macrophages and tumor cells by up to 80% in a dose-dependent manner. The anti-LFA-1 MAb reduced (between 55 to 79%) the number pf P815, LSTRA, or EL-4 tumor cells bound to trypsin-sensitive structures on bacillus Calmette Guerin activated macrophages. The inhibition appeared selective, because a F(ab')$_2$ fragment of anti-MAC-1 did not inhibit such binding. Inhibition of tumor cell capture could be observed as soon as 15 min after the onset of the cell-cell interaction between activated macrophages and tumor cells. Optimal inhibition occurred when both tumor targets and macrophages were precoated with the MAb.

Although P815, LSTRA, EL-4 and BW5147 tumor cells all expressed LFA-1, only the first three but not BW5147 cells were bound by activated macrophages. Furthermore, endotoxin-pulsed macrophages elicited by thioglycollate broth expressed the LFA-1 antigen but did not exhibit selective tumor cell capture. Finally, anti-LFA-1 inhibited the development of weak into strong binding. Taken together, the results suggest that LFA-1 molecules can participate in the interaction between activated macrophages and neoplastic cells.

Strassmann, G., *Springer, T. A.*, Sommers, S. D., and Adams, D. O.

The Journal of Immunology 136 (**11**): 4328-4333, June 1, 1986.

Other support: U. S. Public Health Service and R. J. Reynolds Industries, Inc.

From the Deparment of Microbiology-Immunology, Duke University Medical Center, Durham, NC.

VII. *Metabolic Studies*

THE MECHANISM OF ACTION OF LYMPHOKINES. IX. THE ENZYMATIC BASIS OF HYDROGEN PEROXIDE PRODUCTION BY LYMPHOKINE-ACTIVATED MACROPHAGES

The purpose of this study was to elucidate the biochemical basis of the enhanced hydrogen peroxide (H_2O_2) production by guinea pig peritoneal macrophages (MP) cultured in lymphokine (LK)-containing medium. The markedly augmented H_2O_2 generation by these cells, demonstrable by the horseradish peroxidase (HRP)-catalyzed oxidation of phenol red, is distinguished by its lack of dependence on a second stimulus. We demonstrate that H_2O_2 production is truly spontaneous and is not caused by a stimulant present among the H_2O_2 assay reagents. The principal candidate for such a role was HRP type II (a mixture of five isoenzymes) that was reported to be capable of eliciting an oxidative burst in MP. Four distinct HRP isoenzymes that were found incapable of provoking an oxidative response were nevertheless adequate for demonstrating H_2O_2 production by LK-activated MP. Blocking the MP receptor for mannose by the addition of mannan to the assay system resulted in enhanced detection of H_2O_2 by low concentrations of HRP type II and by three out of four HRP isoenzymes. Treatment of MP with LK-containing medium for 72 hr did not result in a significant change in the activity of cellular superoxide dismutase (SOD) compared with MP cultured for the same length of time in control medium.

By using the specific inhibitor of copper, zinc-containing SOD, sodium diethyldithiocarbamate (DDC), and the universal SOD inhibitor, sodium nitroprusside, we found that the predominant enzyme in guinea pig peritoneal MP is probably manga-

nese-containing SOD. Incubation of LK-activated MP with nitroprusside resulted in almost total inhibition of H_2O_2 production and a simultaneous switch to superoxide (O_2^-) liberation. Similar exposure to DDC had no effect. These data indicate that H_2O_2 produced by LK-activated MP is derived exclusively by enzymatic dismutation of O_2^- mediated by a manganese-containing SOD. The increase in spontaneous H_2O_2 production induced by LK is therefore secondary to augmented O_2^- production that occurs at a cellular location where O_2^- is accessible to SOD.

The enzymatic basis of the enhanced oxygen radical production was investigated by determining the kinetic parameters of the O_2^--forming NADPH oxidase of resting LK-treated MP in a cellfree system in which O_2^- production was induced by sodium dodecyl sulfate. The K_m for NADPH and the V_{max} of the enzyme of LK-treated MP were not different from those of the enzyme of MP incubated in control medium. We conclude that LK treatment of MP does not modulate the NADPH oxidase itself but, most likely, a process related to activation of the enzyme.

Freund, M. and *Pick, E.*

The Journal of Immunology **137**(4):1312-1318, August 15, 1986.

Other support: A. Plesch Research Foundation.

From the Laboratory of Immunopharmacology, Department of Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

THE MACROPHAGE-MEDIATED REGULATION OF HEPATOCYTE SYNTHESIS OF ANTITHROMBIN III AND α_1-PROTEINASE INHIBITOR

Antithrombin III (ATIII) is an anticoagulant protein which binds and inactivates thrombin and other serine proteinases. Little is known about regulation of its synthesis. We confirm that ATIII is synthesized by isolated rat hepatocytes and that its synthesis is not altered by direct feedback of its complexes with proteinases. Neither is hepatocyte synthesis of ATIII altered by supernatants from macrophages cultured in the presence of ATIII-proteinase complexes. However, culture of macrophages with fibrinogen fragment D results in production of a factor(s) in the macrophage supernatants which stimulates hepatic fibrinogen synthesis, as previously described, and also stimulates the synthesis of ATIII and α_1-proteinase inhibitor. Synthesis of albumin and rat α_2-macroglobulin is not altered. Culture of macrophages in the presence of bacterial endotoxin also results in release of a factor(s) into the medium which stimulates the same changes in hepatocyte protein synthesis. These results show for the first time a mechanism by which synthesis of ATIII can be regulated during coagulation and fibrinolysis.

Hoffman, M., Fuchs, H. E., and *Pizzo, S. V.*

Thrombosis Research **41**:707-715, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology, Biochemistry and Surgery, Duke University Medical Center, Durham, NC.

VIII. *Epidemiology*

GENETIC-ENVIRONMENTAL INTERACTIONS IN CHRONIC AIRWAYS OBSTRUCTION

To examine smoking and genetic factors in relation to airways obstruction, cross sectional data were analyzed on 1,787 white non-patient adult participants in a genetic-epidemiological study of airways obstruction (AO), defined as one-second forced expiratory volume (FEV1) less than 68% of forced vital capacity (FVC). Interaction was examined between smoking and each of four factors previously found to be related to AO: alpha-1 antitrypsin (PiZ allele), ABO blood groups (A antigen), ABH non-secretor status, and first degree relationship to a chronic obstructive pulmonary disease or lung cancer patient. Multiple linear regression was used to test for interaction and adjust mean FEV1 (as a per cent of FVC) and prevalence of AO for age, sex, socioeconomic status, coffee and alcohol intake. Statistical interaction was observed between smoking (measured in pack-years) and two genetic factors (presence of blood A antigen and the family history). At higher pack-years levels, those individuals with the A antigen or the family history, but especially those with both factors, had a much lower mean FEV1/FVC% and a much higher prevalence of AO than expected based on a simple additive model. On the other hand, there was no interaction between smoking and PiZ allele, or smoking and ABH secretory status. The findings suggest a possible interaction between cigarette smoke and the airways of individuals with blood group A antigen and familial lung disease. The findings also emphasize the role of genetic-environmental interactions in chronic diseases of multifactorial etiology.

Khoury, M. J. *et al.* (*Cohen, B. H.*)

International Journal of Epidemiology **15**(1):65-72, 1986.

Other support: Lebanese National Council for Scientific Research.

From the Department of Epidemiology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

MILK DRINKING AND POSSIBLE PROTECTION OF THE RESPIRATORY EPITHELIUM

In a Hopkins investigation, detailed interviews as well as spirometry were obtained on 2,539 non-patient adult participants. The interviews included questions regarding smoking habits, family history, socioeconomic status, respiratory symptoms, certain dietary factors, and beverage consumption. For the analyses of risk factors, all patients were excluded from the original study population, which consisted not only of several groups of patients along with their relatives, but also neighborhood controls, teachers and other groups. Thus, only those subjects over 20 years of age who were not ascertained on the basis of their own health status were considered. Chronic bronchitis (CB) was identified by the report of cough and phlegm production for three or more months per year for two consecutive years. Results of this study showed that milk drinking was inversely associated with CB in this genetic-epidemiologic study of chronic lung disease risk factors at The Johns Hopkins Medical Institutions. This finding is of particular interest in view of the well-documented association of vitamin A

deficiency with inflammatory epithelial changes as well as the recent reports suggesting a role of vitamin A in decreased risk of neoplastic changes in the lung.

Tockman, M. S., Khoury, M. J. and *Cohen, B. H.*

Journal of Chronic Diseases **39**(3):207-209, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Department of Environmental Health Sciences and Department of Epidemiology, The Johns Hopkins University, Baltimore, MD.

CIGARETTE SMOKING CHARACTERISTICS OF A SMALL POPULATION OF VOLUNTEER BLOOD DONORS

As part of a three-year study of the effects of cigarette smoking on blood components, demographic data was obtained from healthy volunteer smokers responding to bulletin board notices of solicitation. In addition to age and sex, the type (brand) and number of cigarettes smoked were recorded for each smoker on forms developed to assure collection of data in a uniform and reliable manner. Analysis of the date at completion of the study period revealed that a majority of smokers use filtered versus non-filtered cigarettes (85 vs. 4; $P \leq .05$, Z-test); of the filtered variety, a majority were non-menthol versus menthol (60 vs. 29; $P \leq .05$, Z-test). The 1984 Federal Trade Commission values for each brand's CO, tar and nicotine per cigarette were multiplied by each corresponding donor's reported number of cigarettes per day to estimate total (maximum) CO, nicotine and tar exposure per day; average daily exposures for all smokers were 23 ± 2.43 (SEM) cigarettes per day, 275 ± 20.2 mg CO, 270 ± 21.2 mg tar and 18 ± 1.3 mg nicotine. Males and females were not significantly different. Smokers over age 35 smoked a larger number of cigarettes per day (26.8 ± 2.1 vs. 20.5 ± 1.5; $P \leq .02$, t-test) and thus had higher smoke component exposures. There was no age difference in non-filter cigarette use. These data showing the same use and types of cigarettes for male and female smokers and increases in the number of cigarettes per day in both groups with increased age contrast with data for larger populations less than 15 years ago.

Beyers, B. J., Bowen, R. J., Panus, P. and *Longenecker, G. L.*

The Journal of Research Communications in Substance Abuse **7**(1 & 2):49-57, 1986.

From the Department of Pharmacology, College of Medicine, University of South Alabama, Mobile.

Active Projects

Following is a list of the principal investigators, or institutions, whose projects are underway or were activated in the period since the previous Report, together with the respective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ROBERT H. ABELES, PH.D. *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Development of elastase inhibitors
LEO G. ABOOD, PH.D. *Professor of Brain Research and Biochemistry*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Nicotine transfer-disposition in liver cells
DOLPH O. ADAMS, M.D., PH.D. *Professor of Pathology*, Duke University Medical Center, Durham, NC.	Role and regulation of protein phosphorylation during macrophage activation
IAN Y. R. ADAMSON, PH.D. *Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Cell interactions at the air blood barrier
BURT ADELMAN, M.D. *Assistant Professor of Medicine*, Medical College of Virginia, Richmond.	Effect of fibrinolytic activation on platelet function
KENNETH B. ADLER, PH.D. *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	Airway mucin secretion: effects of products from bacteria associated with chronic bronchitis
JOHN J. ALBERS, PH.D. *Research Associate Professor of Medicine*, University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D. *Professor of Biochemistry*, Harvard University School of Public Health, Boston.	Biosynthesis and processing of PDGF-like polypeptides in human malignant cells in culture
IRIT AVIRAM, PH.D. *Department of Biochemistry*, The Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Isolation, properties and physiological function of neutrophil cytochrome *b*
BERNARD M. BABIOR, M.D., PH.D. *Head, Division of Biochemistry*, Scripps Clinic and Research Foundation, La Jolla, CA.	Studies on the mechanism of activation of the respiratory burst in neutrophils
LAURIE BARCLAY, M.D. *Clinical Director*, The Burke Rehabilitation Center, White Plains, NY.	Tobacco use in Alzheimer's disease
MICHAEL BARANY, M.D., PH.D. *Professor of Biological Chemistry*, University of Illinois, Chicago.	Effect of drugs on membranes of live tissues

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
DAVID W. BARNES, PH.D. *Associate Professor of Biochemistry Biophysics*, Oregon State University, Corvallis	Identification of oncogenes involved in human lung carcinoma
JOEL S. BENNETT, M.D. *Associate Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia	Characterization of the platelet fibrinogen receptor
RICHARD J. BING, M.D. *Professor of Medicine (emeritus)*, University of Southern California School of Medicine, Los Angeles; *Visiting Associate*, California Institute of Technology; *Director of Experimental Cardiology and Scientific Development*, Huntington Medical Research Institutes, Pasadena, CA.	Coronary spasm, cerebral microcirculation
THOMAS R. BROKER, PH.D. *Associate Professor of Biochemistry*, University of Rochester School of Medicine, Rochester, NY.	Cellular transformation by papilloma virus recombinants
DOROTHY L. BUCHHAGEN, PH.D. *Assistant Professor*, State University of New York, Downstate Medical Center, Brooklyn, NY.	Oncogene expression in fetal mouse lung
VINCENZO BUONASSISI, M.D. *Senior Scientist and Deputy Director*, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
JOHN W. BURCH, M.D. *Associate Medical Director*, American Red Cross, Rochester Division, Rochester, NY.	Control of arachidonic acid oxygenation in human platelets
DAVID L. BUSBEE, PH.D. *Professor of Toxicology*, Texas A&M University College of Veterinary Medicine, College Station.	Polynuclear aromatic hydrocarbon transport by serum lipoproteins
EDWARD J. CAMPBELL, M.D. *Assistant Professor of Medicine*, Washington University School of Medicine, St. Louis, MO.	Modulators of inflammatory cell proteolytic activity
DENNIS A. CARSON, M.D. *Associate Member*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mechanism of immune dysfunction after oxidant exposure
DONNA CHAPRONIERE, B.SC., PH.D. Strangeways Research Laboratory, Cambridge, England.	Control of proliferation of cells from the adult human prostate
LAN BO CHEN, PH.D. *Associate Professor of Pathology*, Dana-Farber Cancer Institute, Boston	Studies on human oat cell carcinomas
YUAN-TSONG CHEN, M.D., PH.D. *Assistant Professor of Pediatrics*, Duke University Medical Center, Durham, NC.	Recombinant DNA approaches to assess risk for lung cancer

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
MORDECHAI CHEVION, PH.D. *Chairman*, Institute of Biochemistry, The Hebrew University of Jerusalem, Jerusalem, Israel	The effects of Vitamin C and transition metals on coagulation processes
WILLIAM M. CHILIAN, PH.D. *Assistant Research Scientist*, Cardiovascular Center, University of Iowa College of Medicine, Iowa City.	Pathophysiology of the coronary microcirculation
DOUGLAS BROCK CINES, M.D. *Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia.	Immune injury of human endothelial cells
CURT I. CIVIN, M.D. *Assistant Professor of Oncology & Pediatrics*, The Johns Hopkins Oncology Center, Baltimore, MD.	Biochemistry and function of human granulopoietic antigens
ROBERT A. CLARK, M.D. *Professor of Medicine*, University of Iowa, Iowa City.	Biosynthesis of human neutrophil elastase
GARY A. CLAWSON, M.D., PH.D. *Assistant Professor*, University of California, San Francisco.	Nuclear NTPase and selective RNA splicing / transport
BRIAN L. CLEVINGER, PH.D. *Assistant Professor of Biomedical Science*, Washington University of Dental Medicine, St. Louis, MO.	Role of J segment in V segment expression
CHARLES G. COCHRANE, M.D. *Member, Department of Immunopathology*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease
BERNICE H. COHEN, PH.D. *Professor of Epidemiology*, The Johns Hopkins University, Baltimore, MD.	Airways obstruction and smoking in black and white adults
ROBERT W. COLMAN, M.D. *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Initiation of plasma coagulation and kinin forming systems in man
ROBERT L. CONHAIM, PH.D. *Associate Scientist*, University of Wisconsin, Madison.	Routes of alveolar flooding and clearance
GERALD R. CRABTREE, M.D. *Associate Professor of Pathology*, Stanford University, Stanford, CA.	Retroviral insertion and activation of the IL-2 gene
EVA BROWN CRAMER, PH.D. *Associate Professor of Anatomy and Cell Biology*, Downstate Medical Center, Brooklyn, NY.	Studies of inflammation using an *in vitro* model
CARL E. CRUETZ, PH.D. *Assistant Professor of Pharmacology*, University of Virginia School of Medicine, Charlottesville.	Role of protein phosphorylation in nicotine-induced catecholamine release

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
GIDON CZAPSKI, M.Sc., Ph.D. *Professor of Physical Chemistry*, The Hebrew University of Jerusalem, Jerusalem, Israel	Role of metal ions on superoxide and Vitamin C toxicity in biological systems
IVAN DAMJANOV, M.D., Ph.D. *Professor of Pathology*, Jefferson Medical Center, Thomas Jefferson University, Philadelphia	Developmentally pluripotent human lung cancer stem cells
ALBERT B. DEISSEROTH, M.D., Ph.D. *Professor of Medicine*, Veterans Administration Medical Center, San Francisco	Study of altered alpha globin genes in leukemia and solid tumors
MARTIN E. DORF, Ph.D. *Professor of Pathology*, Harvard Medical School, Boston	Macrophage-like cells involved in immune suppression
PETER H. DUESBERG, Ph.D. *Professor of Molecular Biology*, University of California, Berkeley	Transforming genes of two acute leukemia viruses
REUBEN EISENSTEIN, M.D. *Professor of Pathology*, Mount Sinai Medical Center, Milwaukee, WI	Heparin-binding proteins and endothelial cells
ALVAN R. FEINSTEIN, M.D. *Professor of Medicine and Epidemiology*, Yale University School of Medicine, New Haven, CT	Smoking, detection bias and primary lung cancer
PAUL B. FISHER, Ph.D. *Senior Research Associate*, Department of Microbiology, Columbia University College of Physicians & Surgeons, New York	Chemical-viral interactions in cell transformation
JUDITH ANN FOSTER, Ph.D. *Professor and Chairperson, Department of Biology*, Syracuse University, Syracuse, NY	Involvement of elastin fibers in lung disease
RICHARD B. FOX, M.D. *Assistant Professor of Pediatrics*, Children's Hospital, Boston	Role of glycosaminoglycans in lung edema
IRWIN FRIDOVICH, Ph.D. *Professor of Biochemistry*, Duke University Medical Center, Durham, NC	Control of the biosynthesis of superoxide dismutases
ERROL C. FRIEDBERG, M.D. *Associate Professor of Pathology*, Stanford University, Stanford, CA	Complementing human cells with cloned yeast DNA repair genes
KJELL FUXE, M.D. *Professor of Histology*, The Karolinska Institute, Stockholm, Sweden	Nicotine, catecholamines, and neuroendocrine functions Smoking, dopamine, neuropeptides and models of Parkinson's disease
JACK GAULDIE, Ph.D. *Professor of Pathology*, McMaster University, Hamilton, Ontario, Canada	The mast cell in interstitial pulmonary fibrosis

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
J. BERNARD L. GEE, M.D. *Professor of Medicine*, Yale University School of Medicine, New Haven, CT.	Tissue matrix and phagocyte injury: relative contributions of proteases and oxidants
MICHAEL D. GERSHON, M.D. *Professor of Anatomy and Cell Biology*, Columbia University College of Physicians & Surgeons, New York.	Nicotine effects on neural development: a study of the accessible nervous system of the gut
CHOU ZEN GIAM, PH.D. *Postdoctoral Fellow*, National Institutes of Health, Bethesda, MD.	Immunoglobulin enhancer elements in tissue specific gene expression
GORDON NELSON GILL, M.D. *Professor of Medicine*, University of California, San Diego, La Jolla.	Epidermal growth factor receptor gene in epidermoid carcinoma
GABRIEL C. GODMAN, M.D. *Professor of Pathology*, Columbia University College of Physicians & Surgeons, New York.	Cytoskeletal organization of the endothelial cell in regulation of shape contractility and surface movement
ALFRED L. GOLDBERG, PH.D. *Professor of Physiology*, Harvard Medical School, Boston.	Selective degradation of damaged cellular proteins
WILLIAM E. GOLDMAN, PH.D. *Assistant Professor of Microbiology and Immunology*, Washington University School of Medicine, St. Louis, MO.	Bordetella pertussis tracheal cytotoxin
CHARLES S. GREENBERG, M.D. *Assistant Professor of Medicine*, Duke University Medical Center, Durham, NC.	Transglutaminases and atherosclerosis
MARK I. GREENE, M.D., PH.D. *Director of Immunobiology*, University of Pennsylvania, Philadelphia.	Suppressor cells in syngeneic tumor immunity
NOBUYOSHI HAGINO, M.D., PH.D. *Professor of Anatomy*, University of Texas Health Science Center, San Antonio.	Nicotine on prolactin secretion in development
LINDA M. HALL, PH.D. *Associate Professor of Genetics and Neuroscience*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Genetic differences in nicotine sensitivity in *Drosophila melanogaster* strains
RONALD G. HARVEY, PH.D. *Professor of Organic Chemistry*, The University of Chicago.	Novel anticarcinogenic coumarins and flavones
HENRY D. HOBERMAN, M.D., PH.D. *Professor*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Reaction of aldehydes contained in cigarette smoke with hemoglobin
ROBERT M. HOFFMAN, PH.D. *Assistant Professor of Pediatrics in Residence*, University of California School of Medicine, San Diego, La Jolla.	Methionine dependence, methylation and organic transformation

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
RICHARD L. HUGANIR, PH.D. *Assistant Professor*, The Rockefeller University, New York.	The nicotine acetylcholine receptor: regulation by protein phosphorylation
HAROLD P. JONES, PH.D. *Assistant Professor of Biochemistry*, University of South Alabama, Mobile.	Calcium-dependent regulatory proteins and neutrophil activation
MICHAEL KARIN, PH.D. *Associate Professor of Medicine*, University of California School of Medicine, San Diego, La Jolla.	Isolation and characterization of a heritable fragile site on human chromosome
MORRIS J. KARNOVSKY, M.B., B.CH. *Shattuck Professor of Pathological Anatomy*, Harvard Medical School, Boston.	The molecular basis of pulmonary surfactant secretion by type II pneumocytes: studies in intact cells and a cell-free system
SIMON KARPATKIN, M.D. *Professor of Medicine*, New York University Medical Center, New York.	The role of platelets in tumor cell metastases
ROBERT W. KARR, M.D. *Assistant Professor of Medicine*, University of Iowa, Iowa City.	Development and differentiation of normal and leukemic monocytes
SHIRLEY L. KAUFFMAN, M.D. *Professor of Pathology*, State University of New York, Downstate Medical Center, Brooklyn, NY.	Oncogenes in chemical carcinogenesis
HEINZ KOHLER, M.D., PH.D. *Director*, Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.	Multi-targeting with hybridomas on tumor cells
MARKKU KOSKENVUO, M.D. *Professor and Chairman*, Department of Public Health Science, University of Helsinki, Helsinki, Finland.	The Finnish Twin Cohort Follow-up Study
ROBERT H. KRETSINGER, PH.D. *Professor of Biology*, University of Virginia, Charlottesville.	Crystallographic study of drug-calmodulin complexes
JAMES T. KURNICK, M.D. *Associate Pathologist*, Massachusetts General Hospital, Boston.	Lung cancer study of the *in situ* inflammatory response
JOSEPH LEIGHTON, M.D. *Professor of Pathology*, Medical College of Pennsylvania, Philadelphia.	Atypia and neoplasia of stratified epithelium in gradient culture
MICHAEL R. LIEBER, M.D., PH.D. Laboratory of Molecular Biology, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Bethesda, MD.	Site specific recombination of antigen receptor genes
VALERIE K. LINDGREN, PH.D. *Guest Researcher*, National Cancer Institute, Bethesda, MD.	Viral and cellular factors controlling papillomavirus transcripts

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ZVI LIVNEH, PH.D. *Scientist*, The Weizmann Institute of Science, Rehovot, Israel.	Mechanism of S.O.S. error-prone repair
RICARDO V. LLOYD, M.D., PH.D. *Assistant Professor of Pathology*, University of Michigan, Ann Arbor.	Analysis of pituitary neoplasms with monoclonal antibodies
JOSEPH D. LOCKER, M.D., PH.D. *Assistant Professor of Pathology and Biochemistry*, University of Pittsburgh School of Medicine.	DNA methylation in neoplasia
RONALD J. LUKAS, PH.D. *Director, Laboratory of Neurochemistry*, St. Joseph's Hospital and Medical Center, Phoenix, AZ.	Influences of nicotine on neuronal expression of acetylcholine receptors
JAN M. LUNDBERG, M.D. *Assistant Professor of Pharmacology*, The Karolinska Institute, Stockholm, Sweden.	Sensory neuropeptides and smoke-induced irritation in the respiratory tract
HENRY T. LYNCH, M.D. *Professor and Chairman*, Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Omaha, NE.	Genetic and biomarker studies of cancers of the respiratory tract, pancreas and urinary bladder
RICARDO B. MACCIONI, D.SC. *Assistant Professor*, University of Colorado Health Sciences Center, Denver.	Regulation of microtubule assembly in normal and transformed cells
HOWARD S. MAKER, M.D. *Associate Professor of Neurology*, Mount Sinai School of Medicine, New York.	Nicotine action on brain neurotransmitters and in an animal model of Parkinson's disease
RICHARD A. MARKHAM, M.D. *Assistant Professor of Medicine and of Microbiology and Immunology*, The Jewish Hospital of St. Louis, St. Louis, MO.	T cell-mediated immunity to *Pseudomonas aeruginosa*
WALLACE L. McKEEHAN, PH.D. *Senior Scientist*, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Endocrine control of human endothelial cell regeneration
EDGAR F. MEYER, JR., PH.D. *Associate Professor*, Texas A&M University, College Station.	Structural studies of elastase
STELLA MITRANI-ROSENBAUM, PH.D. *Professor of Virology*, Hebrew University-Hadassah Medical School, Jerusalem, Israel.	Molecular analysis of human genital papilloma virus
DAVID A. MOSCATELLI, PH.D. *Research Assistant Professor*, New York University Medical Center, New York.	Angiogenic factor-endothelial cell interactions
FERID MURAD, M.D., PH.D. *Professor of Medicine and Pharmacology*, Stanford University, Stanford, CA, and *Chief of Medicine*, Veterans Administration Medical Center, Palo Alto, CA.	Mechanism of nitric oxide activation of guanylate cyclase Role of cyclic GMP in smooth muscle relaxation

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
CHRISTOPHER MURLAS, M.D. *Assistant Professor of Medicine*, University of Cincinnati, Cincinnati, OH.	Electromechanical properties of airway muscle
JAY A. NADEL, M.D. *Professor of Medicine, Physiology and Radiology*, Cardiovascular Research Institute, University of California, San Francisco.	Mechanisms of airway hyperreactivity
MOON H. NAHM, M.D. *Assistant Professor of Pathology*, Washington University, St. Louis, MO.	Development of human B Cells
SUSAN NAYLOR, PH.D. *Associate Professor of Human Genetics*, The University of Texas Health Science Center, San Antonio.	Molecular and genetic analysis of small cell lung cancer
DONALD J. NELSON, PH.D. *Associate Professor of Chemistry*, Clark University, Worcester, MA.	Calmodulin interactions with target proteins and synaptic vesicles
JANET M. OLIVER, PH.D. *Professor of Pathology*, University of New Mexico School of Medicine, Albuquerque.	Regulation of the membrane oxidase of human polymorphonuclear leukocytes
F. WILLIAM ORR, M.D. *Associate Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Role of local factors in pulmonary metastasis
YOSHIO OSAWA, PH.D. *Head*, Department of Endocrine Biochemistry, Medical Foundation of Buffalo, Buffalo, NY.	Aromatase inhibitors in cigarette smoke and tobacco
MARY D. OSBAKKEN, M.D. *Assistant Professor of Anesthesia and Biochemistry/Biophysics*, University of Pennsylvania, Philadelphia.	^{31}P NMR study of cardiac metabolism in health and disease
BEVERLY PAIGEN, PH.D. Children's Hospital Medical Center of Northern California, Oakland.	Mapping genetic determinants of atherosclerosis susceptibility
ISRAEL PECHT, PH.D. *Professor of Chemical Immunology*, The Weizmann Institute of Science, Rehovot, Israel.	The role of Ca^{++} ions in basophil and mast-cell degranulation
DENNIS R. PETERSEN, PH.D. *Professor of Pharmacology*, University of Colorado School of Pharmacy, Boulder.	Implementation of the isolated perfused liver to study nicotine metabolism and metabolic interactions
DANIEL E. PETTIJOHN, PH.D. *Professor of Biochemistry/Biophysics*, University of Colorado, Denver.	Role of specific cell surface carbohydrates in the development of human squamous lung carcinoma
EDGAR PICK, M.D., , PH.D. *Professor of Immunology*, Tel Aviv University, Tel Aviv, Israel.	The biochemical basis of enhanced oxygen radical production by lymphokine-activated macrophages

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
JAMES M. PIPAS, PH.D. *Assistant Professor*, University of Pittsburgh.	Oncogenes active in colon cancer
SALVATORE V. PIZZO, M.D., PH.D. *Associate Professor of Pathology*, Duke University Medical Center, Durham, NC.	Protease regulation and cellular metabolism
JULIA M. POLAK, D.SC., M.D. *Senior Lecturer in Histopathology*, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.	Investigation of the role of regulatory peptides in human lung disease
RAMI RAHAMIMOFF, M.D. *Professor of Physiology*, Hebrew University-Hadassah Medical School, Jerusalem, Israel.	Humoral effects of small cell carcinoma of the lung on neuromuscular transmission
LOLA M. REID, PH.D. *Associate Professor*, Albert Einstein College of Medicine, The Bronx, NY.	*In vitro* assay prediction of metastatic potential
JOHN E. REPINE, M.D. *Assistant Director*, Webb-Waring Lung Institute; *Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	Basic mechanisms of lung injury from inhaled oxidants
WARD RICHARD RICE, M.D., PH.D. *Assistant Professor*, University of Cincinnati, Cincinnati, OH.	Neuropeptide hormone regulation of surfactant secretion
NADIA ROSENTHAL, PH.D. Children's Hospital, Boston, MA.	Regulatory factors in muscle gene expression
HARRY RUBIN, PH.D., D.V.M. *Professor of Molecular Biology*, University of California, Berkeley.	Adaptive *vs.* selective effects of alkylating agents
UNA S. RYAN, PH.D. *Research Professor of Medicine*, University of Miami School of Medicine, Miami, FL.	Interactions of hormones with cells of the pulmonary vascular wall
JEFFREY D. SAFFER, PH.D. *Associate Staff Scientist*, The Jackson Laboratory, Bar Harbor, ME.	HMG proteins in chromatin
AZIZ SANCAR, M.D., PH.D. *Associate Professor of Biochemistry*, University of North Carolina, Chapel Hill.	Nucleotide excision repair
BRAHMI P. SANI, PH.D. *Head, Protein Biochemistry*, Southern Research Institute, Birmingham, AL.	Selenium-binding proteins
REGINA M. SANTELLA, PH.D. *Associate Professor of Medicine and Environmental Sciences*, Columbia University, New York.	Development of monoclonal antibodies to carcinogen-DNA adducts

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
B. V. RAMA SASTRY, D.Sc., Ph.D. *Professor of Pharmacology*, Vanderbilt University School of Medicine, Nashville, TN.	Maternal smoking and blood concentrations of amino acids in umbilical arteries and veins
	Influence of nicotine on the release of acetylcholine in the human placenta and its implications on fetal growth
H. WILLIAM SCHNAPER, M.D. *Adjunct in Pathology*, The Jewish Hospital of St. Louis, MO.	Biology of the lymphokine, soluble immune response suppressor (SIRS)
CHARLES H. SCOGGIN, M.D. *Head*, Division of Clinical Applications; *Associate Professor of Medicine*, University of Colorado Health Sciences Center, Denver.	The somatic cell genetics of lung cancer
DAVID W. SCOTT, Ph.D. *Professor of Immunology*, University of Rochester, Rochester, NY.	Immune response to modified self-regulation of murine B lymphoma growth
ROBERT E. SCOTT, M.D. *Professor of Pathology*, Mayo Clinic and Foundation, Rochester, MN.	Commitment control and carcinogenesis in normal, preneoplastic and malignant human epithelial cells
HENRY SERSHEN, Ph.D. *Research Scientist IV*, Neurochemistry Division, Nathan S. Kline Research Institute, Ward's Island, New York.	Development of an animal model of Parkinson's disease
JERRY W. SHAY, Ph.D. *Associate Professor*, University of Texas Health Science Center, Dallas.	Role of cytoplasmic elements in the induction and suppression of tumorigenicity
ISAIAHU SHECHTER, Ph.D. *Senior Lecturer in Biochemistry*, The George S. Wise Faculty for Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Effect of thiols and disulfides on cholesterol metabolism
KENDALL A. SMITH, M.D. *Professor of Medicine*, Dartmouth Medical School, Hanover, NH.	Dissection of the eukaryotic DNA replication pathway
STEVEN S. SMITH, Ph.D. *Assistant Research Scientist*, Beckman Research Institute of the City of Hope, Duarte, CA.	Selectivity of DNA methylation in normal and oncogenically transformed cells
MOHAN SOPORI, Ph.D. *Assistant Professor*, University of Kentucky Medical Center, Lexington.	Cigarette smoke-induced alteration of immune response
TIMOTHY A. SPRINGER, Ph.D. *Assistant Professor of Pathology; Chief*, Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute, Boston.	Studies of macrophage subpopulations and differentiation using monoclonal antibodies
ERIC J. STANBRIDGE, Ph.D. *Associate Professor of Microbiology*, University of California, Irvine.	Transfer of specific individual human chromosomes to recipient cells

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
THOMAS P. STOSSEL, M.D. *Chief*, Medical Oncology Unit, Massachusetts General Hospital, Boston	Functional anatomy of the lung macrophage
FLEUR L. STRAND, PH.D. *Professor of Biology*, New York University, New York	Prenatal and postnatal effects of nicotine and ACTH peptides on neuromuscular development and motor behavior in rats
MAKOTO TAKETO, M.D., PH.D. *Associate Staff Scientist*, The Jackson Laboratory, Bar Harbor, ME.	Gene regulation in teratocarcinoma stem cells
D. LANSING TAYLOR, PH.D. *Professor of Biology*, Carnegie-Mellon University, Pittsburgh	Chemotaxis of macrophages
JOSEPH CHARLES TAYLOR, PH.D. *Associate Research Scientist*, City of Hope Research Institute, Duarte, CA.	Ceruloplasmin abnormality in chronic obstructive pulmonary disease
JOHN A. THOMPSON, PH.D. *Associate Professor of Pharmaceutical Chemistry*, University of Colorado School of Pharmacy, Boulder.	Chromatographic separation and comparative metabolism of d- and l-nicotine
WAYNE M. TREBBIN, M.D. *Nephrologist*, Roger Williams General Hospital, Providence, RI	The effects of renal function on nicotine metabolism
EMIL R. UNANUE, M.D. *Chairman and Professor*, Department of Pathology, Washington University School of Medicine, St. Louis, MO.	Physiopathology of normal and activated macrophages
HAROLD E. VARMUS, M.D. *Professor of Microbiology and Immunology*, University of California, San Francisco.	Functional analysis of cellular oncogenes activated during tumorigenesis
PETER K. VOGT, PH.D. *Professor and Chairman*, Department of Microbiology, University of Southern California, Los Angeles.	New *ONC* genes from acute retroviral leukemias
HELEN VAN VUNAKIS, PH.D. *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Purification and properties of a soluble NAD(P) glycohydrolase isolated from the sponge, *M. prolifera*
PETER N. WALSH, PH.D. *Professor of Medicine*, Temple University School of Medicine, Philadephia.	Interaction of platelets with coagulation factors IX and X
PETER A. WARD, M.D. *Professor and Chairman*, Department of Pathology, The University of Michigan, Ann Arbor.	Oxygen-derived free radicals, immune complexes and tissue injury
GEORGE WEINBAUM, PH.D. *Assistant Chairman for Research*, Department of Medicine, The Graduate Hospital, Philadelphia.	The role of peptide methionine sulfoxide reductase in human lungs: a possible defense against protein oxidation and elastin degradation in smokers

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
SAMUEL B. WEISS, PhD. *Professor of Biochemistry and Microbiology*, The University of Chicago.	Sequence modifications in viral DNA by benzo(a)pyrene metabolites
HOWARD G. WELGUS, M.D. *Assistant Professor of Medicine*, Jewish Hospital at Washington University Medical Center, St. Louis, MO.	Human macrophage collagenase and collagenase inhibitor
MICHAEL J. WELSH, M.D. *Assistant Professor of Medicine*, University of Iowa College of Medicine, Iowa City.	Mechanisms controlling ion transport in airway epithelia
ÅKE WENNMALM, M.D. *Professor and Chairman*, Department of Clinical Physiology, University of Gothenburg, Gothenburg, Sweden	Nicotine as inhibitor of prostaglandin formation localization of the inhibitory step and characterization of the cardiovascular implications
ALEXANDER S. WHITEHEAD, D.Phil. *Assistant Professor of Pediatrics*, Children's Hospital, Boston.	Mouse serum amyloid P component: a model acute phase reactant for the study of inflammation at the molecular level.
PAUL V. WOOLEY, III, M.D. *Professor of Medicine and Pharmacology*, Georgetown University Medical Center, Washington, DC.	Effects of chemical carcinogens upon gene loci in the pancreas
STANLEY J. YACHNIN, M.D. *Professor of Medicine and Chief*, Section of Hematology/Oncology, The University of Chicago Medical Center.	Models for the pathogenesis of atherosclerosis. A: Biological effects of oxygenated sterol compounds. B: mevalonic acid and cholesterol biosynthesis and the biosynthesis and regulation of cell growth
DONALD A. YOUNG, M.D. *Professor of Medicine*, University of Rochester, Rochester, NY.	Papilloma virus proteins and cell transformation

Grantees of Completed Projects

Following is a list of the principal investigators, or institutions, whose projects have been completed prior to the period covered in this Report. Several of the individuals named are deceased. The titles and affiliations listed were those in effect at the time the work was in progress.

MARIO D. ACETO, PH.D.
Associate Professor of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond.

CLARENCE M. AGRESS, M.D.
Associate Clinical Professor of Medicine, University of California Medical Center, Los Angeles.

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ANTHONY P. AMAROSE, PH.D.
Instructor in Obstetrics and Gynecology, The Albany Medical College of Union University, Albany, NY.

E. T. ANGELAKOS, M.D., PH.D.
Professor of Physiology, Boston University School of Medicine, Boston.

D. MURRAY ANGEVINE, M.D.
University of Wisconsin School of Medicine, Madison.

JOSEPH C. ARCOS, D.SC.
Professor of Medicine, Tulane University School of Medicine, New Orleans, LA.

ALAN K. ARMITAGE, PH.D.
Research Director, Hazleton Laboratories Europe, Harrogate, North Yorkshire, England.

MARILYN S. ARNOTT (RASCO), [illegible]
Assistant Biologist and Assistant Professor of Biology, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston.

THOMAS M. AUNE, PH.D.
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Professor of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia.

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Associate Professor of Medicine, Columbia University College of Physicians & Surgeons, New York.

OSCAR J. BALCHUM, PH.D.
Hastings Professor of Medicine, University of Southern California School of Medicine, Los Angeles.

SAMUEL BALK, M.D., PH.D.
Pathologist, New England Deaconess Hospital, Boston.

FREDERIK B. BANG, M.D.
Professor and Chairman, Department of Pathobiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

A. CLIFFORD BARGER, M.D.
Robert Henry Pfeiffer Professor of Physiology, Harvard Medical School, Boston.

BRODA O. BARNES, M.D., PH.D.
Professor (Affiliate) of Physiology, Colorado State University, Fort Collins.

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Associate Professor of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.

T. C. BARNES, D.SC.
Research Scientist, Philadelphia State Hospital.

CARL G. BECKER, M.D.
Associate Professor of Pathology, Cornell University Medical College, New York.

R. FREDERICK BECKER, PH.D.
Associate Professor of Anatomy and Director, Laboratory of Perinatal Science, Duke University Medical Center, Durham, NC.

RALPH S. BECKER, PH.D.
Professor of Chemistry, University of Houston.

BENJAMIN BELL, M.D.
Director Emeritus, Normative Aging Study, Veterans Administration Outpatient Clinic, Boston.

SAMUEL BELLET, M.D.
Director, Division of Cardiology, Philadelphia General Hospital, Philadelphia.

BARUJ BENACERRAF, M.D.
Fabyan Professor and Chairman, Department of Pathology, Harvard Medical School, Boston

WILLIAM F. BENEDICT, M.D.
Assistant Professor of Pediatrics, University of Southern California School of Medicine, Division of Hematology and Medical Genetics, Children's Hospital of Los Angeles, Los Angeles

BARBARA J. van den BERG, M.D.
Research Pediatrician, Adjunct Professor in Biostatistics, University of California School of Public Health, Oakland

JOHN A. BEVAN, M.D.
Professor of Pharmacology, University of California School of Medicine, Los Angeles

BUDHDEV BHAGAT, PH.D.
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